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YIELD AND PROTEIN CONTENT OF HARD RED SPRING WHEAT UNDER CONDITIONS OF HIGH TEMPERATURE AND LOW MOISTURE[1]

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INTRODUCTION

This paper reports a statistical study of yield and other characters of hard red spring wheat grown under conditions of high temperature and low moisture, of a light incidence of disease, and on a fertile soil known as Fargo clay. Nearly all the wheats were chosen because of high yield, when grown under certain environmental conditions, and most of them were selected for resistance to stem rust. Relationships were studied by the use of means, standard deviations, and standard errors, and the analysis was continued by a consideration of total and partial correlation coefficients, by coefficients of multiple determination, and by total and partial regression coefficients. An analysis is attempted of a correlative study of yield and protein content.

EXPERIMENTAL PROCEDURE

This experiment, conducted at Fargo, N.Dak. in 1932, was 1 of a uniform series of 18 such experiments in this hard red spring wheat area and consisted of 25 varieties planted in a randomized manner in triplicated and guarded rod rows. At harvest time 3-foot samples were pulled from the guard rows for detailed study. These samples were selected as to uniformity of stand, care being taken that no empty spaces in the rows were included. In the case of three of the varieties the guard rows were planted with sib material and in those cases the 3-foot samples were taken from the center or yield rows. The detailed study upon the seventy-five 3-foot samples included the following items: Total number of plants and of heads and the calculated stooling; the number of fertile, sterile, and total spikelets per spike; the total number of kernels per spike and the number of third or mid kernels in the spikelets for each spike; the weight of the grain for each 3 feet, the weight of grain per spike, and the weight per 1,000 kernels; the plumpness and percentage of yellow berry of the kernels; and finally the percentage of protein. In addition to the laboratory data secured on the 3-foot samples, the usual field notes were taken on the standing crop at and before the time of harvest.

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WEATHER DATA

Enough weather data are presented to aid somewhat in the interpretation of the experimental results. The normal mean and 1932 mean temperature and precipitation data for the 4 months, April to July, are shown in table 1.

TABLE 1.—*Normal mean and 1932 mean temperature and rainfall data for each of 4 months at Moorhead, Minn* [a]

Year	April		May		June		July	
	Mean temperature	Rainfall	Mean temperature	Rainfall	Mean temperature	Rainfall	Mean temperature	Rainfall
	° *F.*	*Inches*	° *F.*	*Inches*	° *F.*	*Inches*	° *F.*	*Inches*
Normal	41	2.18	55	2.89	64	4.05	68	3.43
1932	44	1.06	57	2.05	69	2.16	72	1.36

[a] Data are from the official records of the United States Weather Bureau.

The drought of 1932 was really a continuation of a drought persisting for at least 4 years. The total deficiency in rainfall for the 4 months indicated for the 4 years 1929 to 1932 was upwards of 19 inches. As the subsoil was very dry in 1932, the amount of moisture depended almost entirely on the showers during the growing season. It is worth noting that during 18 days in July the excess in temperature totaled 155° F.

EXPERIMENTAL DATA

The wheats and their yields as determined from the rod rows and from the 3-foot rows are shown in table 2.

TABLE 2.—*Yields of wheat as determined from 3-foot rows and from rod rows*

No.	Variety	Yield (bushels per acre)	
		3-foot row	Rod row
12	Kota × Webster, H-209	46.1	46.4
8	Marquis × Kota, 1656.106	43.4	46.2
21	Marquis × Hard Federation, 649	41.8	44.4
10	Ceres (Marquis × Kota)	41.8	44.3
7	Marquis-Kota × Hope, Ns 2421	42.6	42.9
9	Marquis × Kota, 1658.178	47.9	42.0
19	Minnesota 2340 (Marquis-Iumillo × Kanred-Marquis)	36.0	40.1
16	Minnesota 2315 (Marquis-Iumillo × Kanred-Marquis)	35.2	39.5
6	Hope × Florence, Ns. 2503	44.0	39.3
4	H-44 × Marquis, R.L. 592	36.3	[a] 38.6
2	Hope × Ceres, N.N. 1098	41.9	37.5
1	Marquis	31.8	36.7
20	Marquis × Emmer, H-44	36.9	35.9
5	Pentad × Marquis, R.L. 729	36.2	35.7
11	Kota × Webster, H-151	39.0	35.5
14	Marquis-Emmer × Marquis, Sel. 1280	34.9	35.4
22	Marquis × Hard Federation, 666	37.3	35.0
15	Marquis-Emmer × Kota-Webster, N.N. 1087	41.3	34.1
25	Hope × Reliance, N.N. 1131	31.9	33.8
18	Minnesota 2302 (Marquis-Iumillo × Kanred-Marquis)	36.7	33.3
17	Minnesota 2316 (Marquis-Iumillo × Kanred-Marquis)	34.3	32.7
3	Hope × Ceres, N.N. 1127	26.6	31.4
23	Hope × Reliance, N.N. 1121	31.2	30.8
24	Hope × Reliance, N.N. 1128	28.8	30.4
13	Marquis-Emmer × Marquis, Sel. 1087	32.7	23.8
	Average	37.4	37.0

[a] Below this point odds are 23 to 1 or more against the occurrence by chance of deviations as great as, or greater than, the ones indicated from the highest yield.

All wheats in table 2 are unnamed hybrid selections except two, Marquis and Ceres. The variety Hope, entering into several of the crosses, was produced by McFadden (*5*) [3] by crossing Marquis and emmer. This variety and also H-44 are highly resistant to both kinds of rust and smut.

The resistance of Hope to stem rust is remarkable in that the plant in the mature stage in the field is not appreciably attacked; it must thus be resistant to all physiological forms present. This variety also shows resistance to leaf rust. Smith (*8*) has shown that Hope when seeded in the spring is highly resistant to five physiologic forms of *Tilletia tritici* and *T. levis* but is moderately susceptible to the same forms when planted in the fall. Of the 25 wheats in the nursery 14 had a 14-chromosome wheat parent either as P_1 or P_2. With the exception of Marquis and the two wheats derived from the cross Marquis × Hard Federation, selection has been made for resistance to stem rust. The wheats in this experiment were not affected appreciably by disease except that the yield and bushel weight of the Marquis-Hard Federation cross no. 666 were reduced by stem rust. Injury of this sort to the companion selection no. 649 was decidedly less and probably not at all material. The essential absence of disease in an injurious form simplified the study.

Nearly all the wheats in the experiment were included because of previous promising performance in yield. The great range of yields in the rod rows, from 46.4 to 23.8 bushels, indicates that in the essential absence of losses due to disease some other factor or factors must have been operating to reduce yields of certain varieties. Because of the drought and high temperatures prevailing, it is likely that it is these factors to which one must look for an explanation of certain low yields. This paper presents a study of a series of wheats subjected to high heat and low moisture, factors which exerted differential effects in regard to yield. Yield represents a complex of contributing components.

Aside from those shown in table 2, yields were taken of one of the guard rows. In three instances the guard rows were of sib selections thus bringing an error into the guard-row yields. The guard rows averaged 37.5 bushels per acre, a difference of but one half bushel from the center, or yield rows. The correlation in yield between the center and the one series of guard rows was 0.652. There was no difference in variability of yield between the two series of rows, guard and center. This indicates that adjacent border rows of different varieties had not acted as an upsetting factor in border-row yield. The correlation in yields between the 3-foot rows, taken from the other guard rows, was 0.659 which is essentially the same as the coefficient just cited. The variability in yield of the 3-foot samples was not significantly greater than the similar coefficients from the rod rows. The yields from the 3-foot rows evidently corresponded fairly well with the yields from the longer rows. Most of the data were secured from the shorter rows, and thus yields and other characters are generally in close correspondence.

[3] Reference is made by number (italic) to Literature Cited, p. 147.

The variety Hope and its sibs have shown rather marked susceptibility to hot and dry weather, and this character has been exhibited in a considerable proportion of the offspring. From table 2 it is evident that hybrids from the Marquis-Emmer parentage tend to yield less than the average of the group. The wheats of this particular hybrid origin yield very significantly less than the wheats unrelated to Hope, when averages are compared, in the group under consideration. Ceres and its allies have shown a capacity to produce relatively well under conditions of drought and heat (Waldron (*10*)). Wheats of other relationships are shown among the 25, and this study may be of aid in showing which physical characters are associated with high and low yields under medium drought conditions.

Most of the studies presented in this paper are based upon correlation and regression data. Table 3 shows the averages of groups of 5 of the 25 wheats, arranged in order with respect to yield, and to 9 other characters of greater or less importance. Similar data are presented for the highest-yielding and the lowest-yielding wheats and for the two named varieties, Ceres and Marquis.

TABLE 3.—*Data on 25 wheats segregated into 5 groups and on 4 individual wheats showing means as indicated*

Group no.	Yield per acre	Heads per row	Kernels per head	Mid kernels per head	Weight per 1,000 kernels	Weight of grain per head	Plumpness (scale 8-1)	Height	Date first heads	Protein in wheat
	Bushels	*Number*	*Number*	*Percent*	*Grams*	*Centigrams*		*Inches*		*Percent*
1	44.8	130	27	12.5	33.7	65	5.9	43.2	June 15.8	13.0
2	41.2	136	27	12.2	31.2	58	5.2	42.8	June 17.3	13.3
3	36.7	134	23	5.0	31.3	52	4.7	43.1	June 17.4	14.0
4	34.6	137	24	4.7	29.5	48	4.9	43.5	June 18.4	14.2
5	30.1	137	26	6.2	26.5	43	4.1	43.5	June 19.5	14.0
High [a]	47.9	114	31	18.9	34.7	79	7.3	45.3	June 16.3	11.8
Low [b]	26.6	139	28	7.4	25.7	42	3.3	46.0	June 19.3	14.2
Ceres	41.8	120	30	15.9	32.1	66	7.0	44.3	June 16.7	12.0
Marquis	31.8	125	25	5.1	29.3	48	4.7	44.7	June 19.0	13.7

[a] 1658.178.

[b] Hope×Ceres, N. N. 1122.

The data in table 3 show at a glance some of the relations which will be expressed later by more refined methods. The heads per row and kernels per spike have no particular relationship to yield, but the contrary is the case with respect to the next four characters in order. No relation is found between height and yield, contrary to what is often found, and with regard to date of heading and percentage of protein a negative relationship exists with yield. The foregoing statements apply generally when one considers the two wheats, high and low, and the two varieties Ceres and Marquis.

ANALYSIS OF VARIANCE

The mean, the standard deviation, and the standard error were calculated for 20 items and are presented in table 4 for both variety and replicate analysis. In the first instance 25 variate means are concerned and in the second instance only 3, as there were 3 replications in the experiment.

TABLE 4.—*Statistical constants of wheat from the Fargo regional nursery*

No.	Character	Mean	Standard deviation	Standard error				Number excluded *
				Variety	Replicate	Significant difference		
						Variety	Replicate	
				Percent	*Percent*			
1	Yield, mid row ..... bushels	37.0	6.6	7.0	2.4	7.7	2.7	16
2	Yield, row B ..... do	37.5	6.5	6.2	2.2	7.0	2.4	18
3	Yield per 3 feet, from guard row ..... do	37.4	7.0	7.8	2.7	8.8	3.0	15
4	Weight per bushel ..... pounds	59.5	2.1	1.1	.4	1.9	.7	17
5	Weight per 1,000 kernels ..... grams	30.4	3.9	4.2	1.5	.4	.1	15
6	Yellow berry ..... percent	4.0	5.3	57.1	19.8	6.9	2.4	4
7	Protein ..... do	13.7	1.2	4.1	1.4	1.7	.6	9
8	Plants per 3 feet ..... number	64.5	12.0	6.1	2.1	12.0	4.0	22
9	Spikes per 3 feet ..... do	134.0	18.1	6.5		26.0		10
10	Stooling	2.1	.3	6.0		.4		14
11	Fertile spikelets per spike ..... number	13.5	1.0	2.6	.9	1.1	.4	20
12	Sterile spikelets per spike ..... do	2.3	.5	5.4		.4		21
13	Total spikelets per spike ..... do	15.8	1.0	2.3	.7	1.1	.3	15
14	Kernels per spike ..... do	25.3	2.9	3.9		2.9		20
15	Mid kernels per spike ..... do	2.1	.7	16.8		1.1		24
16	Mid kernels per spike ..... percent	8.1	4.8	12.1		3.6		23
17	Weight of grain per spike ..... decigrams	5.3	1.1	5.9	2.0	.9	.3	24
18	Date of heading (June)	17.7	1.6	2.0		3.0		11
19	Height of plant ..... inches	43.3	1.6	1.0	.3	1.3	.5	22
20	Plumpness of kernel, scale 8-1	5.0	1.7	18.7	5.5	2.3	.8	12

* As outside the limits of significance.

When experimental data are given statistical treatment, measures of value are calculated for the various constants. These measures commonly take the form of the "standard error." The standard error calculated for a mean is based upon the squares of the deviations of the variates from their mean. In Fisher's method of analysis of variance (*3*) the experiment is planned in such a manner that it is possible to remove from the total variability of the experiment all sources of variability other than those due to experimental error. Further, these other sources of variability are analyzed into their components. In the work at hand these components were generally two, one due to varietal differences and one due to replicate differences. Furthermore, in planning such an experiment, the rod rows are arranged in a random manner. This arrangement tends to eliminate any association values which might enter if the arrangement were systematic and thus the validity of the standard error is increased. This method was of especial value in this experiment as it made possible the elimination of variability between the replicates, in the calculation of the standard error, which here was of considerable moment. The last column shows the number of varieties which are outside the limits of significance and which could be safely discarded were reliance to be placed upon this one experiment.

The third replication had a lower yield than the first or second. The differences were just significant between replications 2 and 3, but decidedly significant between replications 1 and 3 for all three sets of yields. The lower yield of replication 3 was evidently due to an increased deficiency of soil water along with the associated modification in fertility. The deficient supply of moisture apparently affected in a significant manner nine distinct characters in addition to that of yield. Where replicate differences were not significant,

blanks are indicated. The means of the second replication show changes in the same direction but are insignificant. Within the experiment, then, it is evident that one portion has been subject to a more severe drought effect than has another. Some of the changes involved are indicated in table 5.

TABLE 5.—*Constants and other values indicating changes from replication 1 to replication 3*

Character	Standard error	Deviation / Probable error	Change in replication values	
	Percent		*Quantity*	*Percent*
Yield per 3 feet.....bushels	2.7	4.7	−4.3	−11.1
Plants per 3 feet.....number	2.1	3.9	+5.0	+8.0
Fertile spikelets per spike.....do	.9	4.4	−.5	−3.7
Yellow berry.....percent	19.8	5.0	−4.0	−70.0
Plumpness, 8 classes	6.5	5.0	−1.5	−26.4
Protein.....percent	1.4	5.9	+1.1	+8.3
Bushel weight.....pounds	.4	5.9	−1.2	−2.0
Height of plant.....inches	.3	6.2	−.7	−1.6
Weight per 1,000 kernels.....grams	1.5	7.5	−3.1	−9.7
Weight grain per spike.....centigrams	2.0	8.9	−8.0	−15.7

The number of plants per 3 feet and the protein content showed increases from the first to the third replication, while the other characters showed decreases. It is not clear why there should be significant differences in stand among the replications, as similar seeding conditions and, apparently, similar soil conditions prevailed at the time of planting. The number of heads per 3 feet also showed increases from replication 1 to 3, but the difference just escaped significance. An initial thicker stand in the third replication might have been carried through to harvest with a lessened yield and with an effect on the other characters of table 4. However, the difference in stand between the two replications corresponds to a difference of but 5.5 pounds, or 7.6 percent, of seed per acre. This difference in rate of seeding would scarcely affect the yield. Recent studies by Hayes, Wilson, and Ausemus (*4*) show clearly that even larger differences in rate of seeding of wheat do not bring about differences in yield. The correlation between stand and yield is -0.219 ± 0.075. About one third of the minus products entering into this correlation come from the three Hope × Reliance wheats. The stand of these wheats is 23 percent above the average while the yield is but 82 percent of the average. The wheats used in seeding were not studied relative to weight of kernel, but it seems probable that the kernels of the Hope × Reliance wheats weighed less than average. The weight per 1,000 of these three wheats when harvested was but 85 percent of the average. It is questionable if a stand as much as 23 percent above the average would have an injurious effect upon yield. If the greater number of fertile culms showed a too-crowded condition, a compensation would develop in the way of fewer fertile spikelets. Only in pronounced overcrowding could one look for a decreased yield per acre. Evidence on this point is presented by R. W. Smith [4] who presents results from a date and rate of seeding experiment at Dickinson, N.Dak. An increase in rate of seeding of 20 percent, from 75

[4] SMITH, R. W. REPORT ON COOPERATIVE CEREAL INVESTIGATIONS AT THE DICKINSON SUBSTATION. 149 p. 1930. (Typewritten report.)

to 90 pounds, resulted in a wholly inappreciable defect in yield of 0.6 bushel, or less than 4 percent, under conditions of drought. Stand counts are not given.

The drier conditions in the third replication showed an effect sufficiently early to reduce the number of fertile spikelets and height of plant. A decrease in plumpness of 1.5 units is associated with an increase in protein of 1.1 percent and a decrease in bushel weight of 1.2 pounds, in a comparison of the first and last replicates. A very marked change came about in the weight of grain per spike, amounting to 9 cg, or 15.7 percent.

CORRELATION DATA

Correlation coefficients were calculated between those characters where causal relationships seemed to be involved. It was particularly desired to learn the various relationships involving yield, and the relationships involving protein content also seemed to be of particular interest. Certain other coefficients were needed in the calculation of partial correlations. Forty-three coefficients are presented in the following tabulation:

Correlation coefficients showing various relationships between yield, protein content, and various other characters

Yield and—		Percent of protein and—	
Weight per bushel	0.438	Percent of yellow berry	−0.706
Weight per 1,000 kernels	.688	Spikes per row	.157
Percent of yellow berry	.492	Percent of mid kernels	−.292
Percent of protein	−.556	Weight of grain per spike	−.586
Plants per row	−.219	Date of heading	.393
Spikes per row	.205	Plumpness	−.615
Stooling	.454	Spikes per row and—	
Fertile spikelets per spike	−.021	Kernels per spike	−.508
Kernels per spike	.271	Percent of mid kernels per spike	−.372
Percent of mid kernels per spike	.483	Weight of grain per spike	−.469
Weight of grain per spike	.742	Date of heading	.065
Date of heading	−.571	Plumpness	.021
Height of plant	.063	Percent of mid kernels per spike and—	
Plumpness	.587	Weight of grain per spike	.695
Weight per bushel and—		Plumpness	.157
Weight per 1,000 kernels	.341	Weight of grain per spike and—	
Percent of yellow berry	.296	Date of heading	−.568
Percent of protein	−.391	Number of kernels per spike	.584
Date of heading	−.178	Plumpness	.521
Plumpness	.664	Plumpness and—	
Weight per 1,000 kernels and—		Date of heading	−.340
Percent of yellow berry	.466	Percent of yellow berry	.549
Percent of protein	−.527		
Spikes per row	−.168		
Kernels per spike	.065		
Percent of mid kernels per spike	.265		
Weight of grain per spike	.722		
Date of heading	−.580		
Plumpness	.630		

The probable errors are not given in the tabulation, but it may be remembered that if the coefficient is as large as ±0.22, it is on the threshold of significance; and if the coefficient is as large as ±0.30, the ratio of the deviation (from zero) to the probable error is 4, thus indicating marked significance. All but 4 of the 14 coefficients concerned with yield are significant, and most of them are distinctly

so. The highest correlation is weight of grain per spike with yield, which is 0.742. Similar results were obtained with spring wheats by Waldron (*10*) and by Sprague (*9*) with winter wheats. Evidently weight of grain per spike is a complex character influenced, for example, by weight of kernel, plumpness, and number of kernels per spike. Partial correlations will lead to a better understanding of these different relationships. One notes a distinctly sharper relationship of yield with mid kernels than with total kernels per spike. This will be discussed later. The correlation of yield with number of heads per row is just below the limit of significance. This is not surprising when one notes the marked negative correlations between heads per row and kernels per spike and also weight of grain per spike.

YIELD AND DELAYED HEADING

The correlation between yield and date of heading is -0.571. The regression of yield on date of heading is -2.4 bushels for each day's delay in date of heading. The total range in date of heading was 5 days. Bridgeford and Hayes (*1*) in their studies with spring wheats at Morris, in southern Minnesota, found a positive correlation of 0.27. Other things being equal, one might look for larger yields to accompany a longer photosynthetic period. But conditions may modify this rule, especially with spring-sown cereals.

The history of the introduction of varieties of common wheat into the spring-wheat area shows that those newly introduced wheats which have become successful usually possessed a shorter life history than the wheats which were supplanted, and the comparative yield trials show that these earlier wheats have had the higher yields, in comparison with the older sorts. This empirical fact has not been given a critical analysis. One object in securing earlier varieties, with Marquis for instance, was to obviate early fall frosts in Canadian regions. Marquis, and the still earlier Reward have been considered favorably because of their presumed capacity to escape the attacks of stem rust. The idea that the introduction of early wheats was an advantage because of their capacity to escape conditions of heat and drought, which generally tend to become more intense with the progress of the season, has generally been neglected. It has been shown by Waldron (*11*) that under conditions of drought, where stem rust has not entered as a disturbing factor, earlier wheats evidently have an advantage in yield.

It is evident, however, that a negative correlation of the sort indicated cannot continue to be linear. The value of an extended photosynthetic period is offset in part by the physical actualities of the season. Evidently there is a golden mean to be secured in a variety whereby maximum yield results will obtain over a series of years, in comparison with either later or earlier wheats. It is believed that the conditions prevailing with respect to the present experiment were fairly typical of an ordinary spring wheat season and this being so, evidently the majority of the wheats were too late in maturing for optimum yield results. It seems fairly evident that one variety may be more efficient in its photosynthetic work than another. In such a case the whole physicochemical complex supporting the photosynthetic activities would need to be well integrated. While it seems rather probable that a situation of this sort holds with Ceres, it is evident that the problem needs to be given critical study.

YIELD AND PROTEIN CONTENT

The correlation of yield to protein content is −0.556. The regression of yield on protein content is −3.4 bushels, which is the deviation in yield in bushels for each percent of deviation in protein. The total deviation in protein among the varieties is 3.4 percent. The correlation between yield and protein content when date of heading is held constant is reduced from the zero order of −0.556 only to −0.439. Such a marked relationship as is indicated by this negative correlation is of much economic importance, as a high protein content is desired by the commercial interests while a good yield is naturally a concern of the producer. Where the regression is as great as is indicated here it is evident that a distinct protein premium on the market would be necessary to recompense a farmer for his high-protein wheat. An occasional crop might be high in both yield and protein, but in a series of crops grown under conditions like the foregoing the above regression would probably hold. While it is recognized that the higher percentage of protein is generally in evidence in the drier years, the matter apparently has been given but little statistical treatment, as references in the literature seem to be meager.

The total correlation value of 0.341 between weight per bushel and weight of 1,000 kernels is reduced to −0.133 when plumpness is held constant. As expected, weight per bushel and plumpness are strongly correlated, and it is not surprising that a significant negative correlation, −0.391, exists between bushel weight and protein content, although this is reduced to a nonsignificant amount when plumpness is held constant. Mangels and Sanderson (7) in a study of miscellaneous samples taken during 4 years, each year being considered individually, found no significant correlation between weight per bushel and protein content. They point out that the lack of correlation they found might be due to the presence of two factors responsible for reduced bushel weight, heat and rust, which act contrarily upon the protein content and that the samples were not sorted with respect to the two differing factors. In the present instance the lower bushel weights, with the exception of one wheat, no. 666, were due to heat and drought injury. This one wheat contributed but little to the product moment.

The correlation of weight per 1,000 kernels to percentage of yellow berry, 0.466, is reduced to a nonsignificant amount when plumpness is held constant. Likewise the coefficient of −0.527 concerned with protein content and weight per 1,000 kernels is reduced to the horizon of significance when plumpness is held constant. The relationship of kernel weight to date of heading shows a negative correlation, −0.580, and this is reduced to only −0.499 when plumpness is held constant. In this experiment the later heading wheats have the smaller kernels irrespective of plumpness. One would scarcely expect this rule to hold generally, especially where optimum conditions are afforded for maturing the grain and when adaptable varieties are grown.

PROTEIN CONTENT

Seven of the nine correlations involving protein content are minus and significant. In only one relationship, where date of heading was concerned, was the correlation positive and significant. With these

wheats, at least, in a season where conditions favor quite fair yields and in the face of rather adverse heat and moisture conditions and with rust injury of little moment, high protein content is gained at the expense of other characters, some of them of marked desirability, such as yield and bushel weight. One notes a high negative correlation, -0.706, between the percentage of protein and the percentage of yellow berry. The presence of yellow berry is one of the indicators of low grade; a wheat must have 85 percent of dark vitreous kernels in order to grade No. 1 Hard.

Mangels (*6*) in a study of numerous milling samples during 4 years, 1922 to 1925, found that the relationship between protein content and percentage of dark kernels varied considerably from year to year and that correlation might be very low. As he worked with a complex of samples, including many which evidently had been affected in starchiness and bushel weight by shriveling due to the rust organism (Mangels and Sanderson (*7*)), and presumably with samples affected by weathering, it is evident that his coefficients might not be comparable to the one given here, where the problem has been rendered more simple by restricting the study to samples of which the protein content and starchiness were conditioned by varietal and growing conditions, and which were not affected appreciably by disease.

In the present case the regression of protein content on percentage of yellow berry is -0.16. Thus a 10 percent deviation in yellow berry would be associated, generally, with about a 1.5 percent negative deviation in protein content. This appears to be a rather sharp reaction. The percentage of yellow berry per variety varied from 0 to 18.7. The third replicate of the experiment, as shown in table 5, had a significantly lower yield and a lower percentage of yellow berry, and a sharp increase in protein content. These differences are all very significant.

A negative correlation of -0.469 exists between spikes per row and weight of grain per spike. A similar correlation of -0.72 was found by Bridgford and Hayes (*1*). In the present instance the regression is such that a deviation of 10 percent from the mean in number of heads leads to a minus deviation from the mean in weight of grain per head of 7.4 percent.

The character of percentage of mid kernels per spike is of particular interest as some of the higher yielding wheats, including Ceres and its two allies, are characterized by a high percentage of mid kernels. This character is evidently derived from the Kota parent as indicated by Waldron (*11*). The correlation between weight of grain per spike and percentage of mid kernels is 0.695, and the regression of grain weight per spike on percentage of mid kernels is 1.6. Thus a deviation of mid kernel percent of three units results in a deviation of 9 percent of weight of grain per head. The grain weight per head, as was pointed out, is strongly correlated with yield.

PARTIAL CORRELATIONS

As yield is probably the most important character of the experiment, partial correlations were calculated to show the relationship of yield to different characters when one or more of the other characters were held constant. The characters considered, besides (1) yield, are (2) spikes per row, (3) percentage of mid kernels, (4) weight per

1,000 kernels, (5) plumpness, and (6) weight of grain per spike. The total correlations, and all the partial correlations where yield is considered as a variable, are given in table 6. The numbers used in the entries in the table refer to the numbered characters just given.

TABLE 6.—*Total and partial correlations involving yield as the variable in all cases* [a]

Yield (1) correlated with—									
Spikes per row (2)		Percentage of mid kernels (3)		Weight per 1,000 kernels (4)		Plumpness (5)		Weight of grain per spike (6)	
	Correlation		*Correlation*		*Correlation*		*Correlation*		*Correlation*
12	0.205	13	0.483	14	0.688	15	0.587	16	0.742
12.3	.473	13.2	.615	14.2	.748	15.2	.595	16.2	.969
12.4	.447	13.4	.430	14.3	.663	15.3	.591	16.3	.645
12.5	.238	13.5	.489	14.5	.505	15.4	.272	16.4	.488
12.6	.933	13.6	−.068	14.6	.328	15.6	.351	16.5	.631
12.34	.702	13.24	.695	14.23	.796	15.23	.626	16.23	.950
12.35	.524	13.25	.644	14.25	.594	15.24	.225	16.24	.931
12.36	.932	13.26	−.002	14.26	.194	15.26	.066	16.25	.952
12.45	.424	13.45	.450	14.35	.469	15.34	.308	16.34	.276
12.46	.927	13.46	.107	14.36	.337	15.36	.349	16.35	.460
12.56	.923	13.56	.056	14.56	.209	15.46	.246	16.45	.476
12.345	.693	13.245	.703	14.235	.664	15.234	.267	16.234	.863
12.346	.927	13.246	.105	14.236	.220	15.236	.099	16.235	.916
12.356	.923	13.256	.021	14.256	.183	15.246	−.006	16.245	.927
12.456	.922	13.456	.153	14.356	.252	15.346	.268	16.345	.230
12.3456	.921	13.2456	.106	14.2356	.210	15.2346	.014	16.2345	.851

[a] The statements concerned with the significance of the coefficients of correlation following the tabulation on page 135 applies likewise to the coefficients of table 6.

The partial correlation coefficients were calculated from 75 variates. This is probably too small a number to give the most satisfactory results, but the individual values for the most part are not single observations. The yields are correlated fairly well with the yields obtained from the two other rows of the replicates. The regression of the 3-foot yields on the rod row yields is 0.7 bushel. The errors in yield can be assigned to both sets of rows; whether the larger measure of errors belong to the 3-foot rows cannot be determined. The standard deviation of the 3-foot rows is 6.6 percent greater than that for the rod rows, and the difference in the two standard deviations is 0.43 ± 0.53.

The variates of some of the other characters are based on means. The characters with respect to the spike were taken on a sample of 50 heads, or nearly that number. Such a character as plumpness would likely not be much modified if additional readings had been made. The marked changes which result when this or that variable is held constant make it evident that the partial correlations are of real significance. For instance, the total correlation of yield and spikes per row is 0.205; but if the weight of grain per spike is constant, then the partial correlation has the high value of 0.933. It is evident that with respect to yield the two components, number of spikes per row and weight of grain per spike, form nearly or quite a closed system. The difference between 1 and the partial correlation value of 0.933 would be the sampling error. A study of table 6 shows how the presence or absence of a character held constant changes the value of a coefficient.

MULTIPLE CORRELATION

In interpreting the partial correlations of table 6 it is important to consider multiple correlation. A multiple-correlation coefficient can be calculated from partial coefficients, according to Yule (*12, p. 248*) by the following formula:

$$1-R^2_{1.23\ \ldots\ n}=(1-r^2_{12})\ (1-r^2_{13.2})\ (1-r^2_{14.23})\ \cdot\ \cdot\ \cdot\ (1-r^2_{1n.23\ \ldots\ (n-1)}).$$

The value of R^2, termed by Ezekiel (*2*) the coefficient of multiple determination, is the value most commonly used, rather than R, and expresses the proportion of the variance of the dependent variable explained by the independent variables under consideration. We may consider the partial correlation $r_{16.24}$. By using the above formula the two values may be calculated:

$$R^2_{1.24}=0.578;$$
$$R^2_{1.246}=0.944.$$

When spikes per row (2) and weight per 1,000 kernels (4) alone are considered, it is apparent that 57.8 percent of the variance in yield is explained and that 42.2 percent remains unexplained. Similarly when weight of grain per head (6) is made a part of the calculation as a third variable, it is found that 94.4 percent of the variance of yield is explained by a consideration of the three variables while but 5.6 percent remains unexplained. It is evident that the addition of the third independent variable increased the explained variance by 36.6 percent. This value may be expressed in relation to the 42.2 percent left unexplained when only the two factors are considered, by dividing 36.6 by 42.2. The quotient, expressed as 86.7 percent, is the additional explanation, expressed on the basis of the original unexplained total of 42.2 percent, brought about by the introduction of the third independent variable. The square root of 0.867, 0.931, is the partial correlation of $r_{16.24}$. The partial correlation coefficient is thus defined in a somewhat different manner.

In studying the correlation of yield and heads per row, one notes the total correlation, 0.205, is comparatively low, but that the value immediately rises when the percentage of mid kernels, the weight per 1,000 kernels, or the weight of grain per head is held constant. The value is exceptionally high in the last instance, the coefficient being 0.933. The effect on the total correlation is inappreciable when plumpness is held constant, or when plumpness is introduced as a constant in addition to other characters held constant. It seems evident from a comparison of the two coefficients, $r_{12.6}=0.933$, and $r_{12.3456}=0.921$, that the factor, weight of grain per head, might be considered a composite of the characters of percentage of mid kernels, weight of 1,000 kernels, and plumpness. Thus if the character, weight of grain per head, is considered, then the three other characters become automatically considered. This becomes apparent in some of the other columns. The total correlation of yield and percentage of mid kernels is 0.483, but when weight of grain per head is held constant the correlation becomes essentially zero. A corresponding decrease in values is observed when the total correlations of yield and weight per 1,000 kernels and yield and plumpness are considered, in comparison with the same correlations when weight of grain per head is held constant.

The coefficient of multiple determination was calculated and found to be $R^2_{1.23456} = 0.944$. This, or 94.4 percent, indicates the proportionate amount of variance in yield, the dependent variable, which was brought about by the five independent variables indicated. It follows that only 5.6 percent of the squared variability of yield remains unexplained after the five factors indicated are taken into consideration.

If the relationship of weight of grain per spike to the three characters, percentage of mid kernels, weight per 1,000 kernels, and plumpness are considered, when weight of grain per spike is used as the dependent variable, $R^2_{6.345} = 80.3$ percent. Evidently in this case considerably the larger portion of the variance of weight of grain per spike is due to these three variables. The matter can be considered from another angle by omitting this character as such and considering its three components and the number of spikes per row.

PARTIAL REGRESSION COEFFICIENTS

The coefficients of partial or net regression were calculated resulting in the equation

$$\begin{aligned}\text{Yield} = {} & 0.183 \text{ spikes per row} \\ & + .713 \text{ percent of mid kernels} \\ & + .978 \text{ weight per 1,000 kernels} \\ & + .628 \text{ plumpness} - 25.83 \text{ bushels}\end{aligned}$$

The regression or b values of the foregoing equation are all significant; the first three very decidedly so. If this equation is used and the appropriate means of table 4 inserted, the calculated yield will equal the mean yield of 37.4. If one uses average values in this equation for some of the characters and a varietal value for some other character the result is of interest for some of the varieties. If the percentage of mid kernels of Ceres (table 3) is inserted instead of the corresponding average value the resultant yield is 42.9 bushels, which is 1.1 bushels higher than the Ceres yield and 0.4 bushel below its calculated yield. By using the weight per 1,000 kernels for Ceres the resulting yield is 39.0 bushels, which is 2.8 bushels below the yield of Ceres. One might conclude from this that the Ceres character of high percentage of mid kernels was more responsible than any of the other characters considered for the rather high yield of this variety. The same statement would hold true for 1658.178, a sib of Ceres. If these same calculations are made for Marquis by inserting first the Marquis percentage of mid kernels and then the Marquis weight per 1,000 kernels, the calculated yield in the first instance is 35.2 bushels, which is 2.2 bushels below the general average, and 3.4 bushels above the Marquis yield. Similarly by using the weight per 1,000 kernels of Marquis the calculated yield comes to 36.3 bushels. Thus the use of weight per 1,000 kernels reduces the yield less from the average than does the use of percentage of mid kernels.

The coefficient of multiple determination, involving the relationship of the four characters given above to yield, is

$$R^2_{1.2345} = 0.783.$$

This coefficient shows the combined importance of the four independent characters in measuring the variance of yield, the dependent

factor. It is evident that 21.7 percent of the variance remains unexplained.

In measuring the relative importance of the different coefficients entering into the equation of partial regression already given, three sets of values are given. The first set consists of the partial correlation squared. A squared correlation coefficient, as previously indicated, is a measure of the importance of the increased variance which can be explained by the consideration of an added variable. The second set consists of the beta coefficients, commonly calculated, described by Ezekiel (*2, p. 183*); these are calculated by the formula

$$\beta_{12.34} = b_{12.34} \frac{\sigma_2}{\sigma_1}.$$

This formula transforms the partial-regression coefficient as expressed in actual values into coefficients expressed in terms of the standard deviations involved. It is thus rendered more comparable. It is possible to break up the coefficient of multiple determination into components which will add up to its total value. These components are called coefficients of separate determination and constitute the third set. The limitations of these coefficients are indicated by Ezekiel (*2, p. 381*). The three sets of values are given in table 7.

TABLE 7.—*Various coefficients indicating how the regression coefficient values may be measured with respect to (2) spikes per row, (3) percentage mid kernels, (4) weight per 1,000 kernels, and (5) plumpness as affecting (1) yield*

Designation	Coefficients		
	Partial correlation (squared)	Beta	Separate determination
12.345	0.480	0.474	0.096
13.245	.494	.493	.234
14.235	.441	.538	.365
15.234	.071	.153	.088
Total			.783

The value 0.096 is much below 0.480 and 0.474, and this is evidently due to the low total correlation between yield and heads per row. There is no positive correlation between heads per row and the other independent variables. The low value when plumpness is the independent variable is not due to the low total correlation with yield but rather to the correlation of plumpness with weight per 1,000 kernels, which is 0.630.

Evidently weight per 1,000 kernels may be of somewhat more consequence in affecting yield than is percentage of mid kernels. That this is not true in all cases was shown with Ceres and 1658.178. The effect of spikes per row upon yield is probably somewhat exaggerated, as indicated in the first two columns where values are 0.480 and 0.474. The negative correlation between heads per row and weight of grain per head is -0.469, and this fact seems to give undue influence to heads per row when the other yield characters are held constant. One suspects that the coefficient of separate determination

may indicate the relative weightings to be assigned to the four characters in their effect upon yield somewhat better than do either of the other two sets.

DISCUSSION

The present work seems to afford a good opportunity to study the effects of drought and heat on wheat yields largely uninfluenced by disease. The drought and heat injuries were fortunately not extreme, and this fact, in connection with a rather marked divergence among the varieties relative to yield, has added further to the value of the experiment. Some of the physical characters studied are evidently quite highly associated with yield as judged by coefficients of correlation. The weight of grain per spike has a marked correlation with yield, equal to 0.969, when spikes per row, or stand, is held constant. An analysis of this character shows that it is made up mainly of the three components, percentage of mid kernels, weight per 1,000 kernels, and plumpness. Comparative plumpness in this experiment was modified mainly by heat and moisture conditions. Differences in plumpness were not truly inherent in the varieties but were the result of environment. It is presumed that the kernels of all varieties would have been of about the same degree of plumpness had optimum moisture and heat conditions prevailed. Weight per 1,000 kernels is obviously affected by plumpness, but besides this there are inherent varietal differences. Apparently yield in this experiment is affected by differences in kernel weight. The total correlation between yield and kernel weight is 0.688, and when plumpness is held constant the correlation is still 0.505, a very significant figure. The total correlation between weight of grain per spike and weight of 1,000 kernels is 0.722, and when plumpness is held constant the coefficient is 0.594. The character, percentage of mid kernels, is affected probably less by the prevailing heat and drought than either of the characters just considered. The total correlation between yield and percentage of mid kernels is 0.483, and this coefficient is not changed appreciably when plumpness is held constant. A confirmation of the foregoing is seen in a comparison of the replicate means. The means for plumpness and weight per 1,000 kernels of the third replication are much smaller, judged by probable errors, than the corresponding means of the first replication (table 5).

Considering the percentage of mid kernels, the difference between the two replicate means is wholly inappreciable. It was shown that number of spikes per row and weight of grain per spike are responsible for 94.1 percent of the variance of yield. Considering only weight of grain per spike as affecting yield the coefficient of determination is equal to 55 percent, or 55 percent of the variance, or squared variability, of yield is due to weight of grain per spike. The weight of grain per spike was analyzed into the three components already discussed. The coefficient of multiple determination, indicating the proportion of variance of weight of grain per spike brought about by the three characters, percentage mid kernels, weight per 1,000 kernels, and plumpness, was found to be $R^2 = 80.3$ percent. This is appreciably less than the value of 94.1 percent given previously, which is the proportional variance of yield explained through the medium of the two factors, spikes per row and weight of grain per spike. It was thought that the number of kernels per spike might

contribute something to the variance of weight of grain per spike. A recalculation of the value of R^2 with these 4 contributing factors, instead of the 3 just given, raises the value of R^2 from 80.3 to 85.0 percent. Thus there still remains an appreciable amount of the variance of weight of grain per spike unaccounted for even though the various contributing factors seem to have been included in the coefficient. The coefficients of determination, the squared values of r of table 6, for these four contributing factors are as follows:

Variance of grain weight per spike brought about by—

(1) Percent of mid kernels	0. 484
(2) Weight per 1,000 kernels	. 521
(3) Plumpness	. 271
(4) Number of kernels per spike	. 341

While the values as given indicate somewhat the relative influence of the 4 factors the comparative values of 3 of these factors, omitting the fourth, are better shown in table 7. It is evident that both percentage of mid kernels and weight per 1,000 kernels are relatively important factors in influencing yield in this experiment.

PROTEIN CONTENT

A negative correlation of -0.556 was shown between yield and protein content. So little work has been done in this field that it is difficult to say whether this particular case is unusual or not. It is known that the protein content of wheat varies greatly with respect to the available nitrogen in the soil, and protein content may be affected more decidedly than yield, as evidenced by this negative correlation. In the present instance the soil may have been lacking in available nitrogen or it may be that certain of the varieties stood in such a relationship to the seasonal conditions that the soil nitrogen was not of first importance in bringing about the higher yields. If available soil nitrogen tended to be a limiting factor in this experiment, it would be desirable to learn whether the lower yields of some of the varieties were conditioned by the deficient nitrogen. Stated in another way, certain of the better yielding wheats might be able to carry on active photosynthesis in the face of a limitation of nitrogen. The later and lower yielding wheats had the higher content of protein. The high protein content of these varieties did not compensate for the low yield in the total amount of protein per acre. If the 6 high-yielding and the 6 low-yielding varieties are considered, the difference in amount of protein per acre, in favor of the high-yielding sorts, is 85 ± 8.5 pounds of protein per acre. The shortness of growing period was evidently only one factor in bringing about the low protein content. When the date of heading is held constant, the correlation between yield and protein content is still -0.439. However, the correlation between the date of heading and the date harvested has only the moderate value of 0.358. The date wheat is fully ripe is more difficult to determine than the date of heading.

Positive correlations have been shown to exist between yield and spikes per row and yield and weight of grain per head. The last-mentioned character has been analyzed into other characters. With a rather strong negative correlation existing between yield and protein content, negative coefficients may be looked for between protein content and those characters contributing to yield. Evidently in the

relationship between protein and percentage of mid kernels, for example, yield is an influencing factor. The correlation values between protein content and yield factors, both total and partial when yield is held constant, are shown in table 9.

TABLE 9.—*Correlation of protein content with the yield factors indicated* [a]

Character	Total	Partial correlations with yield held constant
Number of spikes per row	0.157	0.333
Weight of grain per spike	−.586	−.312
Percentage of mid kernels	−.292	−.032
Weight per 1,000 kernels	−.527	−.240
Plumpness	−.615	−.429

[a] Correlation values greater than ±0.22 are of significance.

The total correlation of yield with spikes per row was only 0.205. The correlation of protein with this character is positive, the value being 0.157, and this value becomes significant when yield is held constant. In all the other cases shown in table 9 the correlation values are negative, both total and partial. The lowest values are percentage of mid kernels, and when yield is held constant the correlation value is essentially 0. In considering weight per 1,000 kernels the correlation with flour protein might have shown a smaller numerical value, for with smaller kernels the percentage of aleurone layer increases. But plumpness enters into the relationship, and this factor may be responsible in part for the negative correlation between weight of kernels and protein. In fact when both yield and plumpness are held constant, the correlation between protein content and weight per 1,000 kernels falls to 0.090. Attention has been called to the negative correlation between yield and protein content. One would look for a decided lessening of this correlation value if yield factors were held constant. The correlation between yield and protein content when spikes per row and weight of grain per head are held constant is −0.195. This value is slightly below the horizon of significance and shows a marked drop from the total correlation of −0.556.

SUMMARY

Twenty-five hard red spring wheats were grown in randomized rod rows at Fargo, N.Dak., during the season of 1932 under conditions of high temperature and low precipitation, and under a light incidence of disease.

The wide range of yields, from 24 to 46 bushels per acre, was due in large measure to conditions of drought and heat. One replication, because of less available moisture, showed a significantly lower yield than did the others, and significant replicate differences were found for nine other characters.

Significant total correlation coefficients were found between yield and 11 of the 14 characters where calculations were made. The largest coefficient, 0.742, involved weight of grain per spike. The two outstanding negative correlations, with yield, are −0.571, where date of heading is involved, and −0.556, with respect to protein

content. Evidently a lengthened period of photosynthetic activity, normally considered desirable for good yields, may be more than offset by injurious end seasonal conditions of low moisture and high temperature. The supplanting of the older, longer period wheat varieties by the newer, shorter period varieties in the spring-wheat area is a practical confirmation of the present findings.

The correlation of yield to spikes per row is 0.205 and to weight of grain per spike is 0.742. The coefficient of multiple determination for these two factors with respect to yield is $R^2_{1.26} = 94.1$ percent. Thus only 5.9 percent of the variance of yield remains unaccounted for when these two characters are considered. The correlation of yield to weight of grain per spike, when the number of spikes per row is held constant, reaches a high value of 0.967. This is the maximum value in this relationship, despite the holding constant of additional characters. Evidently the factors of number of spikes per row and weight of grain per spike, as affecting yield, constitute a nearly or quite closed system.

The character, weight of grain per spike, is analyzed into the three characters percentage of mid kernels, weight per 1,000 kernels, and plumpness. The three individual correlations are: Mid kernels, 0.695; weight per 1,000 kernels, 0.722; and plumpness, 0.521. The coefficient of multiple determination of weight of grain per spike with respect to these three characters is $R^2_{6.345} = 80.3$ percent. Here 19.7 percent of the variance of weight of grain per spike remains unaccounted for.

When yield is analyzed into 4 factors, the 3 just considered and number of spikes per row, the coefficient of multiple determination with respect to yield is $R^2_{1.2345} = 78.3$ percent, leaving 21.7 percent of yield variance not accounted for. If each of the 4 contributing factors is dropped in succession and comparisons are made, it is found that the addition of percentage of mid kernels results in a greater additional explanation of yield variance than any of the other 4 characters, whereas the character plumpness is of least moment in this respect. Other comparisons indicate that weight per 1,000 kernels is of greater moment than the percentage of mid kernels.

The partial regression coefficients of yield on the 4 characters, spikes per row, percentage of mid kernels, weight per 1,000 kernels, and plumpness are all significant, the first 3 very decidedly so. If the regression equation is used with average values except that values of Ceres and its allies are substituted for percentage of mid kernels or weight per 1,000 kernels, the results indicate that the former character contributes more than the latter to yields approximating those of Ceres. A similar calculation involving Marquis indicates that the use of the factor percentage of mid kernels carries the calculated yield farther from the general average and nearer to the actual Marquis yield than happens when the factor of weight per 1,000 kernels is used.

Of the 9 correlation coefficients calculated involving protein content, 7 are minus and significant. Under conditions similar to those in the present experiment, high protein content is secured at the expense of other desirable characters, such as yield and weight per bushel. In only one instance, which involved the date of heading, was the correlation coefficient with protein both positive and significant.

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RUBBER CONTENT OF VARIOUS SPECIES OF GOLDENROD[2]

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INTRODUCTION

The native plants in which rubber has been found in sufficient quantity to justify intensive study are species of *Asclepias*, *Chrysothamnus*, *Parthenium*, and *Solidago*. The field of possible rubber-bearing plants has been only partially explored, and few species have been collected at all seasons of the year and under sufficiently diverse conditions of growth to determine the possible range of rubber content. Little is known as to the effect of cultivation on the rubber content of plants, the present indications being that some plants produce rubber under conditions of active growth, while others produce rubber only when active growth is retarded.

Since the announcement of the late Thomas A. Edison's discovery that goldenrod contains rubber, the goldenrods (*Solidago*) have attracted much attention. Nearly 100 species of *Solidago* have been recognized by botanists in the United States. Some of these are confined to local areas; others are widely distributed and show many variations in different parts of their range. Just as there is a great difference among the species in flower, leaf, and growth characteristics, there is also a great difference in rubber content. Several of the species have shown considerable variation in the rubber content of samples collected in different localities.

MATERIAL AND METHODS

The plants used for anaylsis were collected in 1931 at several localities near Washington, D.C., in the vicinity of Charleston, S.C., and in the northern part of Florida. Whenever sufficient material was available separate analyses were made of the leaves, stems, and roots. In order to obtain information as to the value of the entire plant as it might be harvested with a mowing machine, several plants of each lot with all leaves and stems were made into a composite sample.

In general the method described by Hall and Goodspeed[3] was followed in making the analyses. According to this method the finely ground sample is first extracted with acetone to remove all

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[2] The plants used for the analyses were collected by Mildred Pladeck, of the Division of Seed Investigations, Bureau of Plant Industry, and W. H. Jenkins, of the Division of Cotton, Rubber, and Other Tropical Plants. Botanical identifications were made by Miss Pladeck and S. F. Blake, of the Division of Botany.

[3] HALL, H. M., and GOODSPEED, T. H. A RUBBER PLANT SURVEY OF WESTERN NORTH AMERICA. Calif. Univ. Pubs., Bot. 7: [159]–278, illus, 1919.

resins and fats, and then with benzol to remove the rubber. The process as described by Hall and Goodspeed was modified in that a 25-ml Gooch filtering crucible was used to replace the siphon cup in the Bailey-Walker extraction apparatus and the extraction period was lengthened to 6 hours instead of 3 for each extraction. The 5-g samples were weighed accurately to 0.002 g, and the rubber and resin extracts were weighed to 0.0001 g. The samples were analyzed in duplicate, the agreement between duplicate samples being sufficiently close to consider the rubber percentages accurate to 0.10 percent.

RESULTS OF ANALYSES

RUBBER CONFINED TO LEAVES

While it was impossible to have separate stem and root samples of every lot, in no case was a significant percentage of rubber found in the stems or roots of any plant, and the results of this series of analyses indicate that none of the species examined can be considered of any importance except on the basis of the rubber in the leaves. Since in goldenrod the leaves represent a very small proportion of the plant, only species having a high rubber content in the leaves, together with a large leaf production, would be considered important. Species such as *Solidago minor* and *S. tenuifolia*, which have minute slender leaves and a comparatively large amount of stem, are unimportant even though they rank high on the basis of the rubber content of the leaves. *S. rugosa*, which produces a comparatively large amount of leaves, ran consistently high on the basis of the rubber content of entire plants, although the highest rubber content found was only 1.55 percent.

RUBBER CONTENT OF LEAVES OF DIFFERENT SPECIES OF GOLDENROD

Leaf specimens from 24 species of goldenrod were tested. The rubber content of the leaves of each species in percentage of the total dry weight of the sample is shown in table 1. The number of specimens analyzed, the high and the low percentage if more than one specimen was analyzed, and the mean if three or more were analyzed, are also shown for each species. While a great variation in rubber content was found in the species of which several samples were collected, in general it was possible to grade them on the basis of the rubber in the leaves into species with high and species with low rubber content. Of the 24 species, a sample of *Solidago altissima* had the highest rubber content, 6.34 percent. Samples of 10 other species had a rubber content of more than 3 percent. The lowest percentage was found in *S. squarrosa*, with a rubber content of 0.56, but in both *S. erecta* and *S. racemosa* the rubber content was less than 1 percent.

A high variation in the rubber content of the leaves was found in some high-yielding species, due either to individual plant variation or to habitat or seasonal effect. This variation is sufficient to indicate a real possibility of plant selection on the basis of rubber content and the development of cultural practices based on optimum conditions of rubber production. That some of the species were found to be more variable than others may be due to the fact that some species, being more common than others, were collected under a greater variety of conditions; or it may be that some species are less well established or have been crossed with other species.

TABLE 1.—*Rubber content (percentage of dry weight) of the leaves of various species of Solidago*

Species	Analyses	Rubber content		
		High	Low	Mean
	Number	*Percent*	*Percent*	*Percent*
Solidago altissima L.	12	6.34	1.38	3.45
S. arguta Ait.	1	1.02		
S. bicolor L.	3	1.20	.89	1.03
S. caesia L.	1	2.46		
S. erecta Pursh	1	.80		
S. fistulosa Mill.	4	4.48	2.08	2.99
S. flexicaulis L.	2	3.46	1.47	
S. graminifolia (L.) Salisb.	2	1.07	1.03	
S. juncea Ait.	2	1.38	.93	
S. minor (Michx.) Fernald	5	3.53	2.63	3.00
S. neglecta Torr. and Gray	1	3.36		
S. nemoralis Ait.	2	2.43	2.38	
S. puberula Nutt.	4	2.35	1.20	1.62
S. racemosa Greene	1	.92		
S. rigida L.	1	3.10		
S. rugosa Mill.	7	3.51	2.63	2.95
S. sempervirens L.	3	3.94	2.22	3.02
S. serotina Ait.	5	5.64	2.40	3.92
S. speciosa Nutt.	2	3.12	2.50	
S. squarrosa Muhl.	1	.56		
S. stricta Ait.	1	2.87		
S. suaveolens Schoepf (*S. odora* Ait.)	3	3.31	2.26	2.68
S. tenuifolia Pursh	1	2.91		
S. ulmifolia Muhl.	2	1.84	1.28	

The variation is particularly striking in the case of *Solidago altissima*, a very common species collected from 14 localities. Leaves of 12 of the 14 samples collected were analyzed, the lowest percentage of rubber being 1.38, the highest 6.34, and the mean of the 12 samples 3.45. *S. serotina*, of which 5 samples were analyzed, also showed considerable variation in rubber content, the range being from 2.40 to 5.64 percent. Of the species having leaves with a relatively high rubber content, *S. minor* and *S. rugosa* showed the least variation.

In general, the species grouped together by taxonomists were found to be grouped on the basis of comparative rubber content. A conspicuous deviation from this rule, however, was found in three species, *Solidago graminifolia*, *S. minor*, and *S. tenuifolia*, which usually are classified under the subgenus *euthamia*. *S. graminifolia*, with percentages of 1.03 and 1.07, had a low rubber content, while *S. minor* with a high content of 3.53 percent and *S. tenuifolia* with 2.91 percent were relatively high in rubber.

RUBBER CONTENT OF GOLDENROD AS COMPARED WITH THAT OF OTHER PLANTS

The rubber content of goldenrod compares favorably with that of other native plants. The highest rubber content found in the present investigation was 6.34 percent, but a rubber content as high as 8 percent was found in Edison's experiments.[4] Guayule has been reported to produce as high as 15 percent, but requires a period of 4 years to attain this content.

Hall and Goodspeed [5] found an old sample of rabbitbrush (*Chrysothamnus nauseosus*) with a rubber content of 6.57 percent. These investigators also found an old gnarled plant of *Aplopappus nanus* from near Benton, Calif., with a rubber content of 9.46 percent.

[4] Oral communication.
[5] HALL, H. M., and GOODSPEED, T. H. Op. cit.

Of the native rubber-bearing plants producing rubber in a single season, the highest percentages of rubber have been found in one of the desert milkweeds, *Asclepias subulata*. Hall and Long [6] reported as high as 6.5 percent rubber in young stems. These investigators also found 5.1 percent rubber in one sample of hemp dogbane (*Apocynum cannabinum*). Specimens of other genera have been reported to contain up to 4 percent of rubber.

NONRUBBER CONSTITUENTS IN GOLDENROD

In goldenrod there is, in addition to the rubber, an appreciable quantity of nonrubber substance which can be extracted with acetone. This material consists primarily of resins, although there are also small amounts of fats, oils, and sugars. Little is known of the chemical identity of the resins or of the possible use to which they might be put, although it has been suggested in the case of similar resins from other rubber-bearing plants that they might be used in paint or varnish or in the manufacture of soap. Since significant amounts have been found in several species of goldenrod, the by-product value of the resins is worthy of consideration.

The highest acetone extract found was 26.45 percent in 1 leaf sample of *Solidago minor*. The mean of the 5 samples of this species was 21.03 percent, and the lowest percentage was 16.24. Acetone extracts greater than 15 percent also were obtained from samples of 4 other species. These included 3 of the 4 samples of *S. fistulosa*, 2 out of 4 samples of *S. puberula*, 1 out of 5 samples of *S. serotina*, and the single sample of *S. neglecta* analyzed.

A positive but very small correlation of 0.397 ± 0.070 was found between the rubber content and the resin content of the 68 leaf samples of *Solidago*. It would appear probable, therefore, that in *Solidago* there is a relation between the rubber content and the acetone extract, but further study under controlled conditions will be necessary to determine adequately the extent of this relation.

SUMMARY

Samples of 24 species of goldenrod (*Solidago*) were analyzed, and significant quantities of rubber were found in the leaves of some of the samples. None of the species contained more than a trace of rubber in any portion of the plant except the leaves. The highest rubber content found was 6.34 percent in one sample of *Solidago altissima*. A rubber content greater than 3.50 percent was found also in specimens of *S. fistulosa*, *S. minor*, *S. rugosa*, *S. sempervirens*, and *S. serotina*. These percentages justify the investigation of selection possibilities in relation to rubber content and the development of cultural practices conducive to the formation of rubber.

[6] HALL, H. M., and LONG, F. L. RUBBER CONTENT OF NORTH AMERICAN PLANTS. 65 p., illus. 1931. (Carnegie Inst. Wash., Pub. 313.) Washington, D.C.

INJURY TO PEARS CAUSED BY PAPER LINERS IMPREGNATED WITH SODIUM SILICATE[1]

By D. H. Rose, *senior physiologist*, and J. M. Lutz, *junior physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Pears of all varieties have one or more layers of stone cells just under the skin, and because of the mechanical resistance offered by these cells the pears are very susceptible to damage by rubbing or bruising. Moreover, the skin of pears usually turns dark brown or black after an injury; hence, after the treatment ordinarily given to pears during the picking and packing processes they are likely to show some discoloration when they reach the market. For the last several years, however, a brown spotting has been observed on certain varieties of pears, both in carload shipments just arrived on the market and in cold-storage lots, which is different in some respects from that caused by rubbing or bruising. It is found on fruits that have been in contact with the corrugated paper used for lining the boxes, or with the excelsior-filled pads used in pear boxes to prevent bruising of the fruit. The spotting is brown in color and usually only superficial. In many instances, especially with the Bosc pear, it occurs in bands crosswise of the fruit, corresponding to the corrugations of the paper liner (fig. 1, A). Often, however, it occurs only as an irregular-shaped brown spot one fourth to 1 inch in diameter. The spotting is sometimes found on all the fruits in a box that touch the corrugated paper and may thus involve one third or more of the contents of a box. It has not been seen on pears or the portions of pears that have not been in contact with a corrugated liner or an excelsior-filled pad.

The injury has been reported several times by inspectors of the food products inspection service of the Bureau of Agricultural Economics, United States Department of Agriculture, usually on pears of the Winter Nelis, Bosc, and P. Barry varieties, all of which show more or less russeting of the skin. It has never been found on smooth-skinned pears, although a dark discoloration on pears of the Anjou variety, the result of bruising or rubbing against the paper liner or against other pears, has sometimes been confused with it. A similar injury caused by bruising or rubbing is frequently found on pears of the Bosc variety.

PRELIMINARY EXPERIMENTS

Analyses made in 1926 by T. D. Jarrell, of the Bureau of Chemistry and Soils, United States Department of Agriculture, from pieces of liner material that had been in contact with affected pears, showed the presence of about 1 percent of sodium silicate. A solution obtained by water extraction of pieces of the liner had a definite alkaline reaction. It seemed probable, therefore, that the spotting was caused by the sodium silicate, which had been used as a binder to

[1] Received for publication Mar. 2, 1933; issued August, 1933.

hold the two parts of the corrugated paper together. In order to test this, small pieces of absorbent cotton were dipped in one fourth, one half, and 1 percent solutions of sodium silicate; most of the solution was squeezed out, and the cotton was then laid on unspotted portions of affected Winter Nelis pears. After the fruit had been held in a moist chamber for 24 hours, spots like those originally noted on the pears were found under all the cotton that had been moistened with a silicate solution. Similar spots were produced within 24 hours when moistened pieces of the liner were laid on other pears of the same lot. No discoloration was found under cotton moistened with dis-

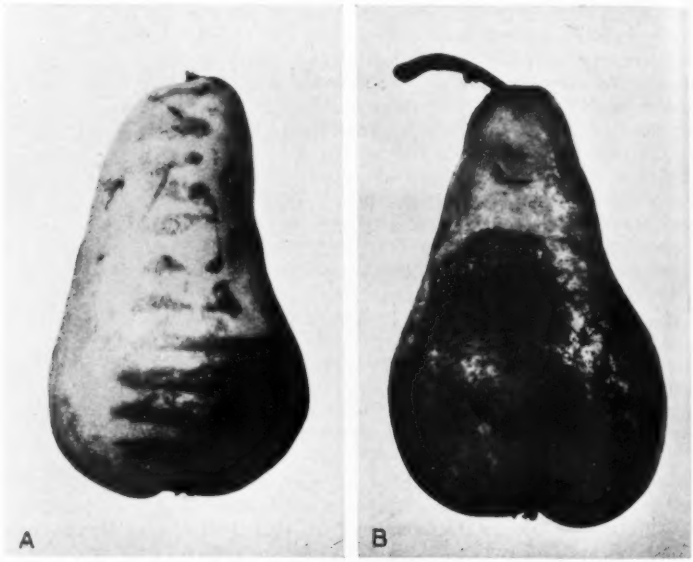

FIGURE 1.—A, Discoloration on a Bosc pear that was in contact with a corrugated paper liner in a commercial shipment. B, Injury produced on a Bosc pear by a 0.1 N solution of sodium hydroxide. This injury was similar to that produced by corrugated-paper liners and alkaline solutions of sodium silicate.

tilled water. It was observed at this time that in all cases the browning was most pronounced on russeted areas.

LATER EXPERIMENTS

After these preliminary investigations no further work was done on the problem until the winters of 1929–30 and 1930–31, when an extensive series of tests was made with six varieties of pears. Seventeen samples of corrugated paper from several sources, 1 sample of excelsior-filled pad, and 5 grades of sodium silicate were used in the tests.

VARIETIES OF PEARS USED

The varieties of pears used in the investigations were P. Barry, Winter Nelis, Bosc, Clairgeau, Bartlett, and Anjou, all of which were grown in Oregon or California. A considerable portion of the skin of P. Barry, Winter Nelis, and Bosc is covered with russet, some speci-

mens being entirely russeted. Clairgeau is a smooth-skinned variety, but the lenticels are conspicuously russeted. Bartlett and Anjou are smooth-skinned, although some specimens of the latter have a russeted area around the blossom end. The region in which the fruit was grown made no significant difference in the results obtained.

No attempt was made to duplicate all conditions with all varieties. Winter Nelis received all treatments. Fruit of the Bosc and Anjou varieties was subjected to most of the test conditions. Bartlett and Clairgeau were used with a few of the paper liners and silicate solutions; P. Barry was used with the paper liners only.

SODIUM SILICATE SOLUTIONS

The five brands of sodium silicate (A, B, C, D, and E) were made up in solutions of five different strengths, namely, 0.1, 0.5, 1, 5, and 10 percent. To test the effect of these, a piece of absorbent cotton about 1 inch square was moistened with the solution and placed on the fruit. The fruit was then placed in moist chambers and most of it was held for 15 days at 40° F. A few lots were held at 32° and 70° to 80° for various lengths of time.

Table 1 shows typical results obtained with solutions of sodium silicate on Winter Nelis, Bosc, and Anjou pears held at 40° F. for 15 days after treatment. The injury consisted of a brown spotting similar to that observed on affected fruit on the market.

TABLE 1.—*Injury produced by various solutions and brands of sodium silicate on pears of three varieties held at 40° F. for 15 days*

Variety	Brand of sodium silicate	Injuries produced by indicated concentrations of sodium silicate solution					
		0.5 percent		1 percent		5 percent	
		Pears	Injury	Pears	Injury	Pears	Injury
		Number		*Number*		*Number*	
Winter Nelis	A	4	None	2	Both moderate	4	All severe.
	B	4	3 severe, 1 moderate.	4	2 severe, 2 moderate.	4	Do.
	C	4	3 moderate, 1 slight.	4	All severe	4	Do.
	D	3	2 moderate, 1 none.	4	2 moderate, 2 slight.	4	Do.
	E	4	1 moderate, 3 slight.	3	All moderate	4	Slight.
Bosc	B	3	1 slight, 2 none.	4	3 severe, 1 moderate.	4	All severe.
	C	3	do	4	None	4	Do.
	E	4	None	4	2 slight, 2 none.	4	Do.
Anjou	B	4	do	4	None	4	2 none, 2 severe on russeted portion only.
	C	4	do	4	do	4	1 none, 3 trace on russeted portion only.
	E	4	do	4	3 none, 1 trace on russeted portion.	4	None.

Solutions of the E brand silicate only were used on the Clairgeau variety. The only injury was a slight discoloration on a russeted fruit, caused by the 5 percent solution.

None of the solutions used had any effect on Bartlett pears at room temperature, about 70° F. No tests were run at other temperatures.

All the concentrations of sodium silicate solutions of each of the five brands were used with Anjou, Winter Nelis, and Bosc pears at room temperature and with Winter Nelis and Anjou at 32° F. No injury was produced on Anjou, and the results with Winter Nelis and Bosc were similar to those shown for these varieties in table 1.

A few drops of each of the five brands of undiluted sodium silicate were poured out on watch glasses and the fruit was placed directly in this liquid. The result was a brown to almost black injury of the skin and flesh, which was severe on Winter Nelis pears and somewhat

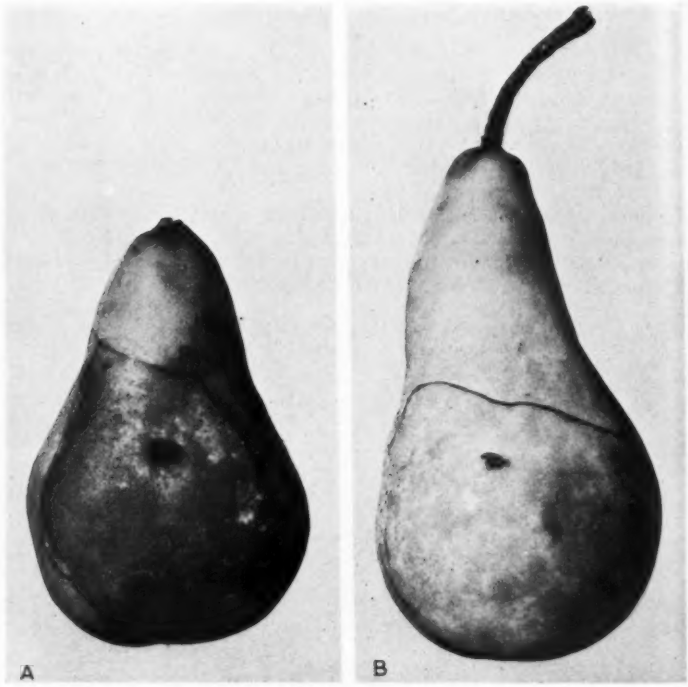

FIGURE 2.—Bosc pears treated with 5 percent sodium silicate: A, Silicate solution not neutralized; B, silicate solution neutralized with sulphuric acid. Areas that were in contact with test solutions are outlined by dark lines.

less so on Anjou. In some cases the discoloration extended into the flesh for a quarter of an inch.

The alkalinity of the various sodium silicate solutions was determined by titrating a 1 percent solution with sulphuric acid, methyl orange being used an an indicator. The results are presented in table 2.

The results of the tests just described furnished some evidence that the discoloration was caused by free alkali in the solution. To obtain further information on this point, enough sulphuric acid was added to the various solutions of sodium silicate used to just neutralize them to methyl orange. The neutralized sodium silicate solutions were

used in the same concentrations, on the same varieties, and under the same conditions as the alkaline sodium silicate solutions previously described. Although the unneutralized solutions caused some injury in concentrations as low as 0.5 percent, and in most instances severe injury to certain varieties, in concentrations of 5 percent the neutralized solutions produced no injury on any of the fruits (fig. 2).

TABLE 2.—*Approximate percentage of alkalinity of sodium silicate solutions*

[Alkali calculated as NaOH, using figures obtained by titrating silicate solutions with 0.1 N H_2SO_4; indicator, methyl orange]

Brand of sodium silicate	0.1 percent solution	0.5 percent solution	1 percent solution	5 percent solution	10 percent solution	Undiluted (calculated)
A	0.02	0.10	0.21	1.05	2.09	20.9
B	.037	.18	.37	1.83	3.67	36.7
C	.015	.08	.15	.76	1.53	15.3
D	.016	.08	.16	.81	1.62	16.2
E	.027	.14	.27	1.37	2.74	27.4

SODIUM HYDROXIDE SOLUTIONS

In order to obtain additional information on the relation of alkalinity to the injury, solutions of sodium hydroxide were applied to pears by means of absorbent cotton in a manner similar to that described for sodium silicate solutions. The results obtained at 40° F. are shown in table 3. The injury produced could not be distinguished from that produced by the sodium silicate solutions, paper liners, or the seams of excelsior-filled pads (fig. 1, B). Dilute acid solutions produced typical acid injury, which was easily distinguished from the injury caused by alkali.

TABLE 3.—*Injury produced by solutions of sodium hydroxide of different normalities on pears of four varieties held at 40° F. for 15 days*

Normality of solution	Winter Nelis		Bosc		Anjou		Clairgeau	
	Pears	Injury	Pears	Injury	Pears	Injury	Pears	Injury
	Number		*Number*		*Number*		*Number*	
0.3 N (1.2 percent)	4	Very severe.	4	2 severe, 2 moderate.			3	Slight on russet portion only.
.2 N (0.8 percent)	4	Severe	3	Moderate	4	Slight on russet portion only.		
.1 N (0.4 percent)	4	do	4	2 severe, 2 moderate.	4	None	3	2 none, 1 trace on russet portion only.
.03 N (0.12) percent	4	2 severe, 2 moderate.	2	1 severe, 1 trace.	4	do	3	None.
.01 N (0.04 percent)	4	do	4	1 trace, 3 none.				

EXCELSIOR-FILLED PADS

Excelsior pads covered with paper are used to some extent in pear boxes as a protection against bruising, especially with tender varieties. The edges of the paper are lapped and fastened together with an

adhesive, usually sodium silicate, and the seams thus made may cover a quarter of the area on one side of the pad.

Moistened pieces of these seams produced injury on Bosc, Winter Nelis, and P. Barry pears (fig. 3, B). This injury was similar to, but usually more severe than, that produced by paper liners. The alkalinity of the seam, determined as previously described for the paper liners, was 2.35 percent, calculated as NaOH. A water extract of the excelsior caused no discoloration of the pear skin.

CORRUGATED-PAPER LINERS

Pieces of corrugated-paper liners about 1½ inches square were moistened with distilled water and placed in moist chambers and pears were laid on them. Only enough water was used to make the

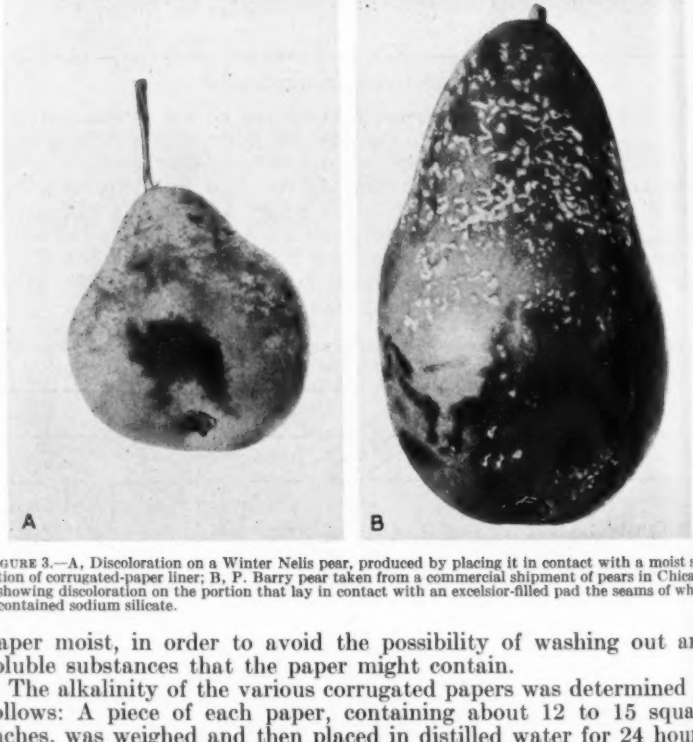

FIGURE 3.—A, Discoloration on a Winter Nelis pear, produced by placing it in contact with a moist section of corrugated-paper liner; B, P. Barry pear taken from a commercial shipment of pears in Chicago, showing discoloration on the portion that lay in contact with an excelsior-filled pad the seams of which contained sodium silicate.

paper moist, in order to avoid the possibility of washing out any soluble substances that the paper might contain.

The alkalinity of the various corrugated papers was determined as follows: A piece of each paper, containing about 12 to 15 square inches, was weighed and then placed in distilled water for 24 hours. The resulting solution was then titrated with 0.1 normal sulphuric acid, methyl orange being used as an indicator. The alkalinity by weight was calculated as sodium hydroxide.

The amount of discoloration caused by different samples of corrugated-paper liners at 40° F. is given in table 4. The duration of the treatment was 15 days.

TABLE 4.—*Discoloration produced on pears of 4 varieties by contact with corrugated-paper liners for 15 days at 40° F.*

Paper liner no.	Adhered with—	Alkalinity expressed as NaOH	Winter Nelis		Anjou		Bosc		Clairgeau	
			Pears	Injury	Pears	Injury	Pears	Injury	Pears	Injury
		Percent	*Number*		*Number*		*Number*		*Number*	
1	Sodium silicate	0.91	4	1 none, 3 moderate	4	None	3	1 severe, 2 moderate	3	None.
2	Flour dextrin paste	.08	4	None	4	do	3	None		
3	do	.09	4	do	4	do	3	Moderate		
4	Sodium silicate	1.06	4	Trace	4	do	3	do		
5	do	.83	4	2 severe, 2 moderate	4	3 none, 1 moderate on russet portion only.	3	Slight		
6	do	1.22	3	2 severe, 1 trace	4	None	3	None		
7	do	.53	3	do			3	do		
8	do	.82	3	1 none, 1 moderate, 1 severe.	4	None				
9	do	.49	4	2 none, 1 trace, 1 moderate.						
10	do	1.43	4	1 trace, 3 moderate	4	None	3	None		
11	do	.79	4	3 none, 1 trace	4	do				
12	do	1.60	4	1 moderate, 3 severe	4	do	3	Moderate	3	None.
13	do	.76	4	None	4	do	3	None		
14	Flour dextrin paste	.95	4	do	4	do				
15	Sodium silicate	.73	4	3 trace, 1 moderate	4	do				
16	do	.76	4	3 moderate, 1 severe	4	3 none, 1 trace on russet portion only.	3	Trace	3	2 none, 1 slight on russet portion only.
17	do	.63	4	Severe	4	None	3	do		
Check	Moist filter paper		4	None	4	do	4	None		

Results similar to those given in table 4 were obtained with Anjou, Winter Nelis (fig. 3, A), and Bosc pears at room temperature (about 70° F.), and with Winter Nelis and Anjou at 32°. Discoloration was produced on P. Barry with paper liner no. 6 at room temperature. None of the papers used produced injury on Bartlett pears.

MOISTURE

To test the effect of moisture in the package during the storage period, Winter Nelis pears were packed in two standard pear boxes with a paper liner that had been found to cause discoloration. The liner in one box was moistened; that in the other box was left dry. About one half of the pears in each box were wrapped in ordinary fruit wrappers. Both boxes were then left at 32° F. for 42 days, after which they were examined. The results are given in table 5.

TABLE 5.—*Effect of moisture in the discoloring of Winter Nelis pears by paper liners, during a storage period of 42 days at 32° F.*

Packing conditions	Pears	Effect on pears at area in contact with paper liners
Liner moist:	*Number*	
Pears wrapped	14	All badly discolored.
Pears unwrapped	13	All badly discolored, slightly more than in wrapped fruit above.
Liner dry:		
Pears wrapped	14	None.
Pears unwrapped	14	1 pear discolored.

REMOVAL OF SPOTTING WITH HYDROCHLORIC ACID

The spotting produced by paper liners was almost completely removed from Winter Nelis pears by allowing the fruit to remain in a 1 percent solution of hydrochloric acid for 1 hour. No apparent removal of the spotting was noticed after 24 hours when pears were placed in water alone.

DISCUSSION OF RESULTS

The typical brown discoloration that has been observed on the market and described earlier in this paper was produced experimentally on russeted varieties of pears by seams of excelsior-filled pads and by corrugated-paper liners when sodium silicate was used as an adhesive. This injury was also produced on russeted pears by solutions of sodium hydroxide and commercial sodium silicate. The injury was not produced by corrugated-paper liners in which flour-dextrin paste had been used as the adhesive instead of sodium silicate. Each of the corrugated-paper liners in which sodium silicate had been used as an adhesive produced the discoloration at least once on either Winter Nelis or Bosc pears during the series of tests.

EFFECT OF RUSSETING

The injury produced seems to be associated with the russeting on the fruit. The sodium silicate solutions, corrugated-paper liners, excelsior-filled pads, and sodium hydroxide solutions produced injury in these experiments on the russeted varieties, namely, Bosc,

Winter Nelis, and P. Barry. The injury on these varieties was most severe on the more heavily russeted fruit. Practically no injury was produced on the smooth-skinned varieties, although a small amount of discoloration was sometimes produced on the russeted areas of Clairgeau and Anjou pears. As heretofore mentioned, the discoloration has never been noticed on the market on smooth-skinned varieties.

Russeting results from a cracking and weathering of the epidermis and an increased development of the corky parenchyma beneath.[2] Fruit russeted as a result of frost injury wilts rather rapidly,[3] which indicates that the skin of a russeted fruit is more permeable to water or water vapor than that of a smooth-skinned fruit.

The brown discoloration on russeted pears probably is caused by the action of the solution on the corky parenchyma or periderm which has been exposed by the disappearance of the epidermis, although it may result from the penetration of the solution into a few layers of cells of the flesh.

EFFECT OF ALKALINITY

The discoloration noted on pears in the various experimental lots of fruit and on those observed on the market had very much the appearance of plant tissues browned by ammonia or other alkalies. It seemed possible, therefore, that the browning produced when certain varieties of pears come in contact with corrugated paper put together with sodium silicate might be caused by free alkali in the silicate. The results of the experimental work described herein make it apparent that the brown discoloration is actually caused in this way. The following facts form the basis for this conclusion:

Although solutions of sodium silicate, in some cases in concentrations as low as 0.5 percent, produced injury, no injury was produced in any case when these solutions were neutralized, even when a 10 percent solution of the silicate was used.

Typical brown discoloration was produced by solutions of sodium hydroxide in concentrations as low as 0.01 N (0.04 percent).

Only the samples of corrugated-paper liners and the seams of excelsior-filled pads that contained sodium silicate produced the discoloration. These were of high alkalinity as compared with the corrugated pads in which some other adhesive was used and by which no discoloration was produced.

Although not conclusive in itself, the removal of the discoloration by a 1 percent solution of hydrochloric acid gives further evidence that the discoloration is due to alkalinity.

EFFECT OF MOISTURE

The humidity within a box of pears is probably high enough at times to cause some of the sodium silicate contained in corrugated-paper liners or the seams of excelsior-filled pads to go into solution and thus be carried to the skin of the fruit, where it produces the discoloration. The moisture probably results largely from condensation produced by the changes in temperature to which boxes of pears are normally subjected. Probably some moisture is also given off by the fruit itself.

HYDROLYSIS OF SODIUM SILICATE

It is realized that when enough acid is added to a sodium silicate solution to render it approximately neutral, the condition established

[2] GARDNER, V. R., BRADFORD, F. C., and HOOKER, H. D. THE FUNDAMENTALS OF FRUIT PRODUCTION. 686 p., illus. New York. 1922.

[3] PADDOCK, W., and WHIPPLE, O. B. FRUIT GROWING IN ARID REGIONS; AN ACCOUNT OF APPROVED FRUIT-GROWING PRACTICES IN THE INTER-MOUNTAIN COUNTRY OF THE WESTERN UNITED STATES. 395 p., illus. New York. 1914.

is probably not permanent because of the hydrolysis of the silicate and the setting free of more alkali in the solution.[4] The extent of this hydrolysis was evidently small, however, during the course of these tests. The fact that solutions of sodium silicate or sodium hydroxide produced the discoloration, whereas "neutralized" solutions of sodium silicate did not, is strong evidence that for the duration of the experiments described there was little or no hydrolysis and consequently little or no release of free alkali into the sodium silicate solution after "neutralization."

SUMMARY

A brown discoloration of russeted varieties of pears is described.

The injury was produced experimentally by placing fruits in contact with corrugated-paper liners and the seams of excelsior-filled pads containing sodium silicate, and by applying to the fruits weak solutions of commercial sodium silicate and of sodium hydroxide. Neutralized solutions of sodium silicate and paper liners put together with some adhesive other than sodium silicate did not produce the injury.

Alkaline substances contained in sodium silicates apparently are the direct cause of the injury as it occurs in commercial lots of fruit.

In many cases there is apparently sufficient moisture within boxes packed with pears to cause some of the alkaline substances contained in paper liners put together with sodium silicate to go into solution and produce the injury.

[4] BOGUE, R. H. THE HYDROLYSIS OF THE SILICATES OF SODIUM. Jour. Amer. Chem. Soc. 42: 2575-2582. 1920.

VITAMINS C AND A IN BLUEBERRIES [1]

By C. R. FELLERS, *research professor*, and P. D. ISHAM, *research fellow, Department of Horticultural Manufactures, Massachusetts Agricultural Experiment Station* [2]

INTRODUCTION

Blueberries are very widely distributed in the United States, Canada, and Newfoundland. *Vaccinium corymbosum*, the high-bush, cultivated type, and *V. pennsylvanicum*, a low-bush, dwarf type, are the common species growing in northern New England, the Canadian Maritime Provinces, and Newfoundland. Several minor species are also of importance in some localities.

Blueberries ripen over a period of several months and keep remarkably well. Fresh blueberries are extensively used as food and are also canned and frozen, and after preservation are used commercially in the bakery, preserve, and jelly industries. Blueberries thus constitute an important item in the diet of large numbers of people over a wide territory.

As no reports on the vitamin content of blueberries had come to the writers' attention, animal feeding tests designed to evaluate this fruit for its vitamin C and A content were accordingly begun in the summer of 1931. The results of these tests are reported in this paper.

EXPERIMENTAL PROCEDURE

VITAMIN C IN BLUEBERRIES

The method of Sherman, La Mer, and Campbell [3] was used, with the exception that the amounts fed were proportioned to weight of the guinea pigs, i.e., a 400-gram animal was fed four thirds as much as one weighing 300 g. The animals were all young and healthy, weighed between 280 and 325 g, and were kept in individual cages. The basal ration consisted of 58 percent equal parts of rolled oats and wheat bran, 30 percent of vitamin C free baked skim-milk powder, 10 percent butterfat, 1 percent each of cod-liver oil and salt. This basal ration and water were kept before the animals at all times.

Three guinea pigs normally were used at each feeding level. At the end of the feeding period all animals were chloroformed and carefully examined for lesions of scurvy. Negative controls died in from 26 to 33 days with an average Sherman scurvy score of 16.

Native western Massachusetts high-bush blueberries, *Vaccinium corymbosum*, were used in both 1931 and 1932 in the tests on fresh blueberries. Since the latter were available for only 85 and 80 days, respectively, in 1931 and 1932, the feeding tests were limited to this period.

Frozen native, high-bush blueberries, *Vaccinium corymbosum*, were from the same lot that was fed fresh in 1932. These were wrapped in cellophane, packed in 1-pound waxed-paper cartons, and frozen at Glouces-

[1] Received for publication Mar. 6, 1933; issued August 1933. Contribution no. 165 of the Massachusetts Agricultural Experiment Station.

[2] The writers are indebted to the Birdseye Laboratories, Gloucester, Mass., for freezing, storing, and shipping the frozen samples from Massachusetts that were used in 1932.

[3] SHERMAN, H. C., LA MER, V. K., and CAMPBELL, H. L. THE QUANTITATIVE DETERMINATION OF THE ANTISCORBUTIC VITAMIN (VITAMIN C). Jour. Amer. Chem. Soc. 44: 165–172, illus. 1922.

ter in the Birdseye multiplate freezer at about −30° F. and stored at 0° for 5 to 8 months. Samples for vitamin assay were shipped weekly with solid carbon dioxide and were still solidly frozen upon arrival at the laboratory. The cartons were held at −0° until just before using.

The Maine blueberries were the low-bush species, probably *Vaccinium pennsylvanicum* and were quick frozen by the Birdseye method at Winslows Mills, Me., during August in 1931 and 1932. No sugar was added. The berries were wrapped in cellophane and packed in 1-pound paraffined-paper cartons. Supplies packed with solid carbon dioxide were sent to the laboratory weekly. The Newfoundland blueberries were also the low-bush type, probably *V. pennsylvanicum*, and were quick frozen by the Birdseye method at St. Johns. They were packaged, stored, and shipped similarly to the frozen blueberries from Maine.

Blueberries were canned in both 1931 and 1932. In 1931 both the native high-bush and the low-bush types were canned. The high-bush native blueberries were blanched for about 30 seconds in boiling water, filled into spring-clamp glass jars, processed in a water bath at 212° F. for 8 minutes, and sealed. The low-bush variety was treated in the same way, except that the jars were vacuum-sealed at a partial vacuum of 25 inches of mercury. These berries were grown on Cape Cod, whereas the high-bush berries were grown in Amherst. In 1932 the high-bush variety from Amherst was again canned. This time the fruit was not blanched but was filled directly into glass jars, covered with a previously boiled hot 10 percent sugar sirup, vacuum-sealed at a partial vacuum of 20 to 22 inches of mercury, and processed at 212° for 8 minutes. The cooled jars had partial vacuums of 15 to 18 inches of mercury.

In every case the guinea pigs ate the fresh, frozen, or canned blueberries. A few animals that disliked the fruit were discarded early in the experiment and new animals substituted for them.

VITAMIN A IN BLUEBERRIES

A preliminary test of the vitamin A content of Maine quick-frozen blueberries was run according to the Sherman and Burtis technic.[4] Depleted rats were fed the vitamin A free diet supplemented by 1 and 3 g of blueberries, but no significant evidence of protection was secured at either level. Although too scanty data were obtained to render these results quantitative, the agreement is such that the conclusion that blueberries contain but a trace of vitamin A, less than 0.3 unit per gram, seems justified.

RESULTS

The results of the experiments on the vitamin C content of blueberries are reported in table 1.

Data on two successive crops of *Vaccinium corymbosum*, high-bush blueberries, show a minimum protective dose of 4 to 5 g. There is little or no loss due to quick freezing. The canning method may be of importance in the retention of vitamin C. For example, the unblanched vacuum-canned blueberries were far superior to the same variety which were canned a year earlier but which were blanched for 30 seconds in boiling water and sealed without vacuumization.

[4] SHERMAN, H. C. and BURTIS, M. P. FACTORS AFFECTING THE ACCURACY OF THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Biol. Chem. 78: 671–680. 1928.

TABLE 1.—*Vitamin C in blueberries*

Source and treatment of blueberries	Year	Animals	Quantity fed	Change in weight	Average survival period	Scurvy score	Remarks
		Number	*Grams*	*Grams*	*Days*		
Native, high-bush, fresh	1931	3	3	24	85	7	Moderate scurvy; experiment terminated in 85 days.
	1931	3	5	110	85	0	Do.
	1932	2	3	40	80	3	Moderate scurvy; experiment terminated in 80 days.
	1932	2	5	349	80	0	Do.
Native, high-bush, frozen	1932	3	7	346	90	0	
	1932	3	14	322	90	0	
Maine, low-bush, frozen	1931	3	4	−120	33	11	Severe scurvy.
	1931	3	8	−109	65	8	
	1932	3	10	149	90	3	Slight scurvy.
	1932	3	14	156	90	1	Trace scurvy.
Newfoundland, low-bush, frozen	1931	3	4	−143	37	12	Severe scurvy.
	1931	3	8	−115	43	11	Do.
Native, high-bush, blanched, canned	1931	3	4	−117	43	11	Do.
	1931	3	6	−115	60	16	Do.
Native, low-bush, blanched, vacuum canned	1931	3	4	−65	39	16	Do.
	1931	3	8	−114	38	14	Do.
Native, high-bush, not blanched, vacuum canned	1932	3	5	360	90	0	No scurvy.
	1932	3	10	311	90	0	Do.
Negative control	1932	3	0	−130	30	16	Severe scurvy.

Too few controlled canning tests were made to justify conclusions relative to the effect of canning methods, yet it is significant that one of the methods used did conserve the vitamin C.

Although the low-bush blueberries, *Vaccinium pennsylvanicum*, were not assayed in the fresh state, nevertheless, inasmuch as quick freezing followed by storage at 0° F. did not injure the vitamin C content of cranberries,[5] strawberries,[6] or the high-bush blueberries, the same process probably caused no loss in the low-bush species.

The results obtained in using the low-bush species indicate considerable variation, both seasonal and regional. The 1932 Maine crop apparently contained more vitamin C than did that of 1931, but even this species is but 25 to 35 percent as active as the high-bush species, the minimum protective portion being 15 to 16 g. There is apparent also a slight difference between the vitamin C potency of the low-bush blueberries grown in Maine and those grown in Newfoundland. The results indicate that the Newfoundland berries are but 75 to 85 percent as potent as the Maine berries.

Only one sample of low-bush blueberries was canned. At the 8-g level, the highest amount fed, very little protection from scurvy was obtained.

SUMMARY

Vaccinium corymbosum, the high-bush blueberry, is a good source of vitamin C. Quick freezing and certain methods of canning did not significantly injure the vitamin C content.

Vaccinium pennsylvanicum, the low-bush blueberry contains only 25 to 35 percent as much vitamin C as does *V. corymbosum*. Only a trace of vitamin A is present in *V. pennsylvanicum*.

[5] FELLERS, C. R. NUTRITIVE VALUE OF CRANBERRIES. Amer. Jour. Pub. Health 23: 13–18. 1933.
[6] ——— and MACK, M. J. Indus. and Engin. Chem. 25: ——— 1933. (In press.)

THE LAW OF DIMINISHING RETURNS IN AGRICULTURE[1]

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INTRODUCTION

A correct understanding of the law of diminishing returns both as to its application, as well as to its statement as a principle, is fundamental in any study designed to measure the effect of increasing units of an input factor upon the output whether this output be in the form of plant growth or animal production.

Immediately following Von Liebig's emphasis of the need by plants of various mineral nutrients necessary for growth, attempts were made to bring under some law the relation existing between added units of any input factor necessary for plant growth and the additional outputs caused by those additional units. Von Liebig developed his law of the minimum, namely, that the productivity of a field is in direct relation to the necessary constituent contained in the soil in the smallest quantity (*8, p. 105*)[2]. In this law he recognized not only the importance of the limiting factor in plant growth but also the necessity of a balanced plant-food supply. The law itself, however, was not correctly stated, and it took the thought and research of four generations of soil chemists to develop a sufficient body of data under carefully worked out control conditions to show (1) that the law as Von Liebig stated it was incorrect and (2) to make a correct statement of the law possible.

DISCUSSION

It was Mitscherlich (*10, 11, 12, 13, 14, 15, 16, 17*) preceded by Mayer (*9*) and Wagner (*20*) who, following years of experimentation, finally restated the law to express the diminishing increments [3] of output with the addition of like units of any input factor. He first experimented with different forms of phosphoric acid fertilizer in pot experiments and showed that as the applications of either of the forms of phosphoric acid were increased the yield also increased, but at a decreasing rate. As the application of monobasic phosphate of lime was increased from 1 to 10 units, for example, the yield increased at the following rate (*19*):

Units applied	Increase in yield per added unit of fertilizer
1. 0	44. 3
2. 5	10. 8
5. 0	7. 2
10. 0	2. 0

[1] Received for publication Oct. 17, 1932, issued August, 1933.
[2] Reference is made by number (italic) to Literature Cited, p. 177.
[3] "Increments" is used to indicate the added output, and "returns", total output.

Later experiments were performed with other fertilizing elements, and these were followed in turn by experiments in which the water and sunlight were varied. The experiment varying the amount of sunlight is interesting. Plants were grown in greenhouse pots shaded by nets varying from 2 to 10 in number for different pots, and the observed growth was compared with full growth in the open. Some of the results are shown in table 1. The increments of growth (or yield) in this instance showed, in general, a decreasing rate in each of the different plants used in the experiment.

TABLE 1.—*Growth increments of plants in greenhouse pots as related to increased intensity of sunlight*

Increase in intensity of sunlight [a]	Increment of growth or yield per unit of added light intensity			
	Oats	Peas	Buckwheat	Lupins
0.025	3.2	1.9	1.6	0.6
.033	3.2	1.4	2	1.2
.044	3.9	1.6	2.2	.9
.063	2.1	.8	1.4	.8
.098	1.7	.7	1.2	.6

[a] In terms of sunlight in the open expressed as 1.00, 0.192 is the least intensity used in the experiment.

It is needless to add to the number of experiments that may be referred to in the United States and in England as well as in Germany to prove the existence of this particular relationship, and it was this type of work which was positively accepted by scientists as proof of the inaccuracy of Von Liebig's statement of the law. It also showed that as any element necessary for plant growth became less (assuming the quantity in the soil to be less than the optimum) the yield was reduced at an increasing rate. Out of this came Mitscherlich's well-known law of the soil.

The next conspicuous development in the theoretical aspects of the law of diminishing increments was to give mathematical expression to the law. Experiments have demonstrated that the cause-result relationships are so certain that they can be shown with mathematical exactitude.[4]

Both Mitscherlich and Spillman, working independently and unknown to each other, showed, through empirical means, that input-output relationships for different plant and animal production assumed a certain form which is expressed by a logarithmic curve. This curve is so constructed that each point on the vertical axis bears the same relation to the maximum vertical rise when expressed in terms of the corresponding point on the horizontal axis as any other point on the axis. If, for example, a unit of any input factor, horizontal axis, results in a yield, vertical axis, of one half the possible maximum which this variant can cause, then the second unit of input will result in the production of one half the remainder; and the addition of a third unit of the input factor will yield one half the remaining possible production which this factor can cause (*1*, *19*).

[4] It should be remembered, however, that results can be predicted with any degree of accuracy only when all the variable factors affecting production or growth are known, and only those are varied whose effects are to be determined.

It is obvious that this curve represents diminishing output per unit of additional input and that exact expression can be given it. In each of these experiments, especially those performed by Mitscherlich, productions were obtained with the variation of a single element of plant growth. Nitrogen, or phosphorus, or sunlight was varied separately, but not two were varied simultaneously. It was in connection with the variation of more than one input factor that Mitscherlich indicated his inability to show and measure results. Baule, however, using some of Mitscherlich's data, deductively arrived at another mathematical expression which when plotted shows a sigmoid or S curve rather than the characteristic logarithmic curve so commonly accepted as the relationship existing between added units of input of any one factor and the corresponding additional outputs.

In working on this problem Baule expressed as 100 the maximum possible yield due to the variation of any single factor (*1*). He then determined the amount of the variable element necessary to produce 50 percent of the maximum, called the Baule unit. If x units of the variable produced 50 percent of the possible maximum, 2x units would produce 50 percent plus one half of 50 percent (remaining production) or 75 percent of the maximum, and 3x units would produce 75 percent plus one half of 25 per cent or 87½ percent, etc. The tabular expressions of the Baule relationships of individual elements of plant growth are shown in table 2.

TABLE 2.—*Baule relationships between individual fertilizing elements favoring plant growth*

Input		Output in terms of maximum output	
Additional	Total	Additional	Total
1	1	½	½
1	2	¼	¾
1	3	⅛	⅞
1	4	1/16	15/16
1	5	1/32	31/32

It was shown that the same relationship would hold for any of the fertilizing elements, so that if the application of nitrogen were varied in one experiment while the application of phosphorus were varied in another it would be possible to make the units of the different kinds of fertilizers of such amounts that the first application in each case would result in an increase in growth equal to 50 percent of the possible maximum growth of the plant; and subsequent applications of the same sized unit would result as indicated in table 2. In other words the curve would be logarithmic.

But what would be the effect of equal doses if a combined unit of nitrogen and phosphorus were applied? It is obvious that the first dose of the combined units of nitrogen and phosphorus would not result in 50 percent plus 50 percent, or 100 percent of the total possible yield. It would be larger than if either had acted alone, but how much larger can be determined only by experimentation. The maximum result of the combined units would again be 100 percent, but this

100 percent seems to bear no known relationship to the "100 percents" representing the maximum possibilities of the respective plant-food elements composing this unit.

If, for example, the greatest possible increase in growth caused by the application of nitrogen to a soil is 40 bushels and the greatest possible increase in yield caused by the application of phosphorus is 30 bushels for the particular soil and climatic conditions in the experiment, it does not necessarily follow that the 100 percent figure for the effect of the combined elements would be 40 plus 30 or 70 bushels. Neither is it logical to expect as small an amount as is received by the application of either of the separate elements alone. Obviously the 100 percent point for the maximum effect of the combined factors would be somewhere between these two points.

Wherever this point is located Baule speaks of it as 100 percent and in order to obtain the relative effect of the simultaneous application of two fertilizing elements he multiplies the proportional yield obtained by one by that of the other. If the first application of nitrogen gives one half of the possible maximum for that element and the first application of phosphorus gives one half of its greatest maximum the effect of the simultaneous application of these two units would be one half times one half, or one fourth of the greatest possible growth, of the combined effects. The second dose of the combined elements would result in three fourths of three fourths or nine sixteenths of the greatest possible growth, and the third dose would result in seven eighths of seven eighths or forty-nine sixty-fourths of the greatest possible growth, of the combined factors. The result of this combination would not be a logarithmic curve but would be a sigmoid or S curve.

The practical significance of this development would be that, whereas the first application (or first unit available for plant growth) of any single fertilizing element or factor of production causes a greater relative growth than any subsequent application of a like unit, the first application of a unit composed of two growth factors would have relatively less effect upon plant growth than would some of the following doses. It necessarily follows from this deductive formulation by Baule that the greater the number of factors of plant growth simultaneously applied, the less will be the relative effect of the first units applied and the greater will be the relative effect of some of the following units (*13*).

It was also brought out by Mitscherlich that the greater the number of variants simultaneously applied, the more delayed will be the point at which the greatest relative effect will take place and the later in the plant growth will the point of diminishing increments occur.

This general relationship is shown graphically in figure 1.

Mitscherlich accepts this as the true relationship between the input and output factors when more than one causal factor comprises the input dose. He shows that it applies to plant growth when the plant development is recorded from a seedling. In his experimentation with sorghum plants in pots under glass the effect of applying equal "quantities of factors of growth" (*13*) composed of several fertilizing elements was shown upon the "accretion" or growth of the plants. Equal growth periods were determined by observing the number of days required for a radish seed to germinate and grow to

the point of formation of its second pair of leaves. He hoped in this manner to equalize variations in growth conditions caused by differences in sunlight, humidity, temperature, etc., so that each growth period would have as nearly equal as possible the amounts of those factors of growth which he wished to hold constant. This resulted in a variation of from 11 to 26 days in the time required for different groups of radishes to develop the same amount of growth. Six of the pots were harvested at each of the successive growth periods until some of the plants had grown nearly 6 months. The amount of growth was obtained by determining the grams of dry substance in each "section of growth", or equal growth period.

When these weights are plotted using equal growth periods as the horizontal or variable axis it shows a typical S or sigmoid curve, which is interpreted by Mitscherlich as proving, or at least demonstrating,

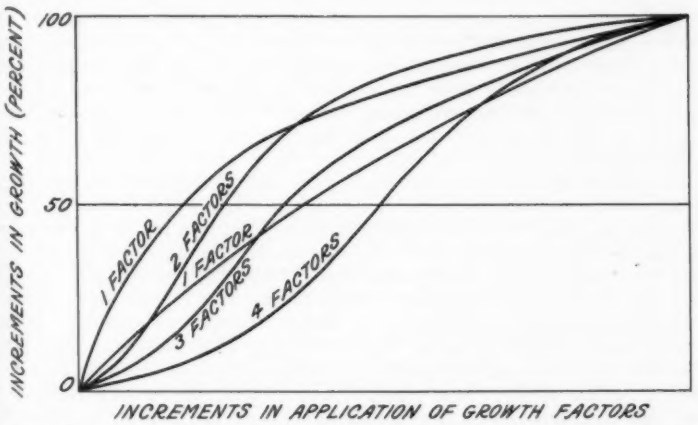

FIGURE 1.—General relationship between the application of various numbers of growth factors and the stage of plant growth at which the point of diminishing returns occurs.

the increasing and decreasing increments of plant growth per unit of input.

Several questions present themselves before this relationship can be accepted as a substitute for the law of diminishing increments or law of physiological relations. It is quite commonly recognized that plant (or even animal) growth is slow in its initial stages of development. Leaf surface for photosynthesis and root area for plant-food absorption are both limited so that only a small amount of plant growth can take place in a given period of time (or growth period). As the plant becomes larger and functional surfaces increase, growth normally becomes more rapid until the plant reaches approximate maturity. At some point in the latter part of the development of the plant the growth is again retarded. This relation of the growth of a plant to the time element has been called the "grand growth curve of plants" and is stated as follows: "Crop growth is accomplished in two principal stages, the first stage being characterized by an increasing rate of growth and the second by a decreasing rate * * *" (*22*, *p. 47*). Growth of this character will take place so long as there are

plant-food elements and other factors of growth in sufficient amounts to mature the plant. Growth will be greater in every stage of its development if larger quantities of growth factors are available, but the increasing and decreasing relationships will continue. This particular phenomenon, or characteristic, is not a function of depleted or changing growth factors but is a function of time alone.

Because of this characteristic of plant growth it will be necessary to call into question any results which presume to measure the effect upon crop production of additional applications of some growth factor, as fertilizers, when they are expressed in terms of growth periods. If the fertilizing elements of nitrogen and phosphorus are being applied in increasing amounts, it will be necessary to determine just how much growth is obtained through the addition of each dose of the combined unit. The effect of the first dose upon plant growth cannot be measured until the plant has taken up all of the dose that is biologically possible. The application of the second dose—or two doses to the second plant—must be treated in a manner similar to the first, and so on until maturity is reached. It is probable that when the results of an experiment of this type are plotted it will be found that the first application of nitrogen and phosphorus gives the greatest growth and that each additional dose results in slightly less growth than does the preceding dose.

Experimentation has shown that in some circumstances initial increments of some plant-food element, as nitrogen, when added to a soil deficient in that element may result in no increase in the availability of that element for plant growth—the added amounts being locked in the soil in the form of salts not readily available for plant growth. This results in small increments in plant growth for the initial applications of the fertilizing element. Although experimentation with the simultaneous application of two fertilizing elements has not been recorded where the results are as indicated above, it may be expected that in certain soil and plant nutrient conditions the same relationship may be observed. No generalization should be made from a few instances of this nature, however, as they are more than offset by experiments showing the greatest increments with initial applications. Recognition must also be given to the difference between application and availability.

It should also be remembered that in each of these generalizations it is presumed that what holds for plant growth also holds for crop production. If diminishing increments are characteristic of plant growth under controlled conditions, it is presumed that diminishing increments will characterize crop production of the same plant when grown in larger areas.

It may be desirable to know whether a small dose of fertilizer will have relatively greater effect upon plant growth when applied at the time of seeding, when the plant is 2 inches high, or when the crop is nearly matured, but this is a different question from that of determining the effect of increasing units of fertilizer upon crop growth or production. It is necessary to know how crop yields will be affected with different growth conditions in order that the generalizations may be effectively applied to the industry of farming; and experiments based upon different stages of plant growth which admittedly are characterized by increasing and decreasing increments must be

properly interpreted with specific reference to this one characteristic if experiments are to be useful in the general production of the crop.

It should be remembered that the method of combining two or more variables is not limited to one mathematical statement. The only premise from which Baule could have developed the sigmoid curve was by assuming that the initial additions of a combination of input factors result in less production than do subsequent additions; while if still more additions of the input factor are made, production will be at a lessened rate.

Had Baule assumed that the initial application of units [5] of any combination of input factors results in the largest relative increase in production, and that subsequent units result in increases but at a decreasing rate, he could have paralleled the logarithmic curve shown by the use of one variable. Feed as well as fertilizer experiments which are sufficiently complete to throw light on this subject show the decreasing output characteristic.

Many experiments have been set up showing the effect of the functioning of the law of diminishing increments, or the law of the soil, as Mitscherlich expresses it, and the results have been generally accepted. But a few questions should be raised relative to some of the conclusions reached.

It has been shown at different times, for example, that this law functions in the application of water to a crop. Widtsoe and Merrill (*21*) show that when varying amounts of irrigation water are applied to wheat the yield of grain per acre-inch of water decreased consistently from 2.02 bushels per inch for 5 acre-inches of water to 0.77 bushel per inch for 50 acre-inches. A crop like corn which has greater yield possibilities in its present stage of plant development showed the same decline in relation to increasing amounts of water used. The yield of this crop ranged from 6.07 bushels per acre-inch of water with the application of 5 acre-inches, to 1.43 bushels per acre-inch with 55 acre-inches of water. Other crops, without exception, showed the same general trend in yields. Sugar-beet yields were reduced from 0.90 ton per acre-inch of water when 5 acre-inches were applied to 0.41 ton when 50 acre-inches of water were applied.

The authors sum up the presentation of the results of their investigations as follows (*21, p. 114–115*):

> There is a great uniformity among the results obtained when different quantities of irrigation water are applied to the representative crops studied in these experiments. In practically every case, increasing the quantity of water increased the total yield of the crop, but in a smaller ratio as the maximum irrigation was approached. The more water that was used, the smaller was the return per acre-inch of water. So large was this decrease of the acre-inch equivalent that a serious waste of water is to be inferred. As farmers become better acquainted with this truth the problem of the best quantity of water to use must ever confront them with more and more force. Shall the irrigation farmer, having money invested in both land and water, attempt to produce the largest yield per acre or the largest yield per acre inch? Is there some point at which acre-yield and acre-inch-yield meet to produce the largest profits?

In this way the problem is thrown open to solution, and the discussion of the law of diminishing increments as a physical principle becomes identified with the economic concept of diminishing returns. How much land should a farmer use with any given quantity of water and with certain price relationships?

[5] "Application" is used here to indicate availability.

Willcox (*22*), in using this same series of data concludes that there is but one way to move in the utilization of land and that "is toward extensive farming." In proof of this contention he uses the yields of wheat obtained when a given amount of water is applied (30 acre-inches in this case) to 1, 2, 3, 4, and 6 acres of land, the total yields being 47, 91, 130, 166, and 226 bushels from the respective acreages. From this he formulates a law of "increasing increments of yield (variable space)", which is the eighth of his laws of plant growth. "When the amounts of the material factors of plant growth are held constant while the areal space is increased, the yield of the crop is increased at an increasing rate" (*22, p. 46*).[6]

The discussion of this law is not very extensive; but if Willcox means that the aggregate production is increased as more units of a variable factor are applied to a fixed factor, it is self-evident and needs no comment. It matters not whether the product is expressed as a series of totals, or in terms of some fixed factor, as the acre-inches of water in this instance, because whenever an increasing total is expressed in terms of a fixed factor the series must show increasing ratios. If, as the writer believes, he is stating the law of increasing increments it is to be questioned either whether he has correctly interpreted the data or whether the conclusion concerning the application of the law to agriculture is correct.

If the production previously mentioned is expressed as yields per acre of land used it will show 47, 45, 43, 42, and 39 bushels per acre as the land area is increased from 1 to 6 acres. This is a typical illustration of decreasing average returns. If, on the other hand, each increment in production is expressed in terms of the added acreage the following series results, as shown in table 3.

TABLE 3.—*Relation between added acreage and production increments on irrigated land, the amount of water used remaining constant*

Total		Increase in—	
Acres	Yield	Acres	Yield per added acre
	Bushels		*Bushels*
1	47	1	47
2	91	1	44
3	130	1	39
4	166	1	36
6	226	2	30

It is here seen that as the land area is increased—the amount of water used remaining constant—production increases but at a decreasing rate per additional unit of input, or acres.[7] This is another illustration of decreasing returns, rather than increasing returns as stated by Willcox.

[6] The graphic presentation used by Willcox to show increasing returns is in error in that he uses acre-inches of water as the horizontal axis (causal or variable factor) rather than the increase in land surface, in acres. Had he plotted the aggregate yields against acres as this descriptive material suggests, he would have observed the diminishing returns characteristic.

[7] It is conceivable that increasing increments will be in evidence if the amount of irrigation water applied is increased from 1 acre-inch, where no crop production will take place, to several acre-inches, where some crop production is obtained. As production increases from zero to something, the rate of increase is infinite and increasing increments must of necessity be evident. It is not so easily conceivable, however, that, after enough water is applied to obtain some production, further additions of equal amounts of water will result in more than proportionate or even proportionate increases in yield.

In making an application of this law to agriculture Willcox (*22, p. 46*) states:

> The law of diminishing increments of yield is characteristic of extensive farming where land is abundant and the other growth factors, as water, fertilizer, labor, etc., are relatively scarce.

Quite the contrary is true.

Where land is relatively free much more land (per unit of other factors) will be used, but even in this instance extra land will show diminishing rather than increasing increments or returns. It would seem that Willcox has confused total returns with returns per unit of the variable and that he does not recognize the implications of the principle of the conservation of the scarce factor.

Another possible interpretation which Willcox may have had in mind is that as the acre-inches of water become less the yield will become increasingly greater per acre-inch of water. The construction of his chart would indicate this possible interpretation as would also his use of the words "space" and "area". It should be remembered, however, that the aggregate amount of water is held constant according to his premise, and that the varying factor is the area over which the 30 acre-inches of water is applied. The variant is not acre-inches of water but acres of land. When 30 acre-inches of water are applied to 2 acres of land instead of 1 the amount of water applied per acre must be one half as much as when applied to the 1 acre, and when the same amount of water is applied to 4 acres it must of necessity follow that but 7½ inches per acre can be used. If the study is for the purpose of determining the effect of different amounts of water on crop production, the land area should be held constant, as was done by Widtsoe and Merrill in their experimentation.

When an irrigation farmer rents land and water rights he, either thinkingly or not, has determined on a working proportion of land to water. Let us assume that, after farming a few years, he finds his yields of wheat, with the applications of the different amounts of water, to be the same as shown in table 3. He also finds that he is limited to a specific quantity of water during the year. Will he use a few acres of land and apply the water at the rate of, say, 30 acre-inches per acre or will he use six times as many acres and apply 5 acre-inches of water per acre?

This question cannot be answered without knowing the rental charge (or cost) per acre of the land, the cost per acre-foot of water, and the price of the crop. The higher the land rent in proportion to the water cost the fewer the acres of land that will be used. If the farmer had to pay $40 an acre for the rent of land and $2.50 per acre-foot for water, a unit consisting of 1 acre of land and 30 acre-inches of water would cost him $46.25. If wheat sold at $1 per bushel he would receive $47, or a net of $0.75 above rent and water cost for each unit consisting of 30 acre-inches of water and 1 acre of land. If, on the other hand, he applied the same amount of water to a unit consisting of 6 acres of land the cost of the unit in this case would be $246.25 while the returns would be but $226, or $19.75 less than rent and water cost for each unit of land and water. This may be an absurd spread of constant and variable costs, but it helps to illustrate the fact that at times it may pay the irrigation farmer to intensify his operations. If the rental charges were but $10 an acre

and other costs and prices were as given above, it would doubtlessly profit the farmer to practice the more extensive type of production. In each instance production is accomplished by decreasing increments per unit of the variable; yet because of the change in relative scarcity (costliness) of one factor the farmer is forced to change the intensity of his farming operations. In no instance is there any indication of increasing increments.

Incidentally if the law of increasing increments of yields as expressed by Willcox does not hold, then his ninth law or "concentration law of growth factors" may well be called into question. This law states that: "In a normal environment the effectiveness of a given quantity of an external factor of crop growth is *inversely proportional* to its concentration per unit of space."

If one unit of fertilizer produces X units of product then, according to the writer's interpretation of this law, 2 units of fertilizer applied to the same area would produce $\frac{X}{2}$ units of the product per unit of fertilizer, or a total of $2 \times \frac{X}{2}$, or X units of product, and the application of 3 units of fertilizer would result in $\frac{X}{3}$ units of product per unit of fertilizer or a total of $3 \times \frac{X}{3}$, or X units. In other words, the greater the application of fertilizer per unit of land the less would be its effect per unit of fertilizer on crop growth. This is obviously in error and cannot be what the author had in mind. If he meant to imply that the influence of each added unit of fertilizer is relatively less than the effect of the preceding unit when applied to the same square foot or acre, this would appear to be a correct statement. It is a restatement of his seventh law, of diminishing increments of yield, however, and should not be set up as a new law.

Another restatement of the law of diminishing increments is found in the eleventh law, action law of growth factors, wherein Willcox (*22, p. 55*) accepts the definite logarithmic formulation of the law as developed by Spillman and Mitscherlich. It is stated as follows:

> When a variable positive growth factor is increased by successive increments the increase in the yield of the crop due to any increment will be proportional to the difference between the total yield produced by the preceding increments and the maximum possible yield * * *.

A third interpretation which may be more in line with the discussion of the author is that, as a given quantity of a growth factor is spread over larger areas, it becomes less concentrated, and the total yield becomes greater per unit of this growth factor which is held constant. This, of course, is true but it is not the statement of a new law. Rather it is a restatement of the truism that the greater the total yield the greater the average yield per unit of any fixed factor.

This principle has also been applied to problems of digestion and metabolism as they affect livestock production. In the different experiments which the writer has reviewed the principle of diminishing increments applies. Planes of feeding work with different classes of livestock, including poultry (*2*), beef (*5, 6, 7*), and dairy production (*3, 4, 18*), all show the general tendency of additional outputs to decrease with added units of any input factor. The authors

have not usually set up their data to show this relationship; but wherever experiments are made with this principle in mind, this study should result not only in pointing the way to greater accuracy in research technic but the material itself should also stand up better under the scrutiny of logical interrogation.

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SOME FACTORS AFFECTING THE ELECTROLYTES OF THE STARCH GRANULES [1]

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INTRODUCTION AND REVIEW OF LITERATURE

The early workers on the constitution of starch regarded it as of purely organic composition [2] (*8, pt. 1*) and of empirical formula $(C_6H_{10}O_5)_n$. While it was recognized that starch could not be prepared free from certain inorganic elements, these were regarded as adsorbed impurites, which could not be washed out by the levigation process used in the preparation of the starch. The structure of the starch granule was extensively studied, and many divergent theories proposed to explain its structure and method of growth. It was generally held, however, that it consisted of two parts, one the insoluble outer envelope called alpha amylose or amylopectin, and the soluble inner part called beta amylose or amylose.

Various workers attempted to remove the mineral elements in the starch by washing it with weak acids. Demoussy (*3*), in 1906, "demineralized" rice starch with weak hydrochloric acid and found that it had the properties of a weak acid, comparable to carbonic acid, and formed compounds with metallic hydroxides and carbonates which were dissociable in water.

Samec (*11, p. 19*), in 1913, proposed the theory that the acidity of starch is due to phosphoric acid, which is combined as an amylophosphoric acid ester and reacts like a dibasic acid. The remaining valences of the phosphoric acid were found to be more or less completely satisfied with cations of the alkalies or alkaline earths. Washing with dilute acid was found to remove the cations but not the phosphoric acid. Autoclaving, however, was effective in splitting off the acid, and a phosphorus-free amylopectin could thus be prepared. The viscous properties of the original material, which had disappeared, could be regained by rephosphorizing.

Of the other mineral constituents, silica is always present in starch in considerable quantities and is considered as an essential part of the starch grain. The question as to whether or not silica also exists as an amylosilicic acid ester has been raised but, according to Samec, has not yet been proven. Traces of manganese, iron, aluminum, and the chloride and sulphate anions are also present, varying according to the extent of purification of the sample. Noncarbohydrate organic groups have recently been shown to be essential constituents of starch from certain genera of plants. Taylor (*16, p. 63*) has found that starch from some of the cereal plants contains fatty acids which are combined with the carbohydrate complex, while Samec (*13*) believes that in wheat starch there is a unit of nitrogen combined with the amylophosphoric acid.

[1] Received for publication Feb. 9, 1933; issued August, 1933.
[2] Reference is made by number (italic) to Literature Cited, p. 190.

Thus, the original concept of starch as a pure carbohydrate has gradually changed. Even though the amounts of these noncarbohydrate materials are small, sometimes only a few hundredths of a percent of the starch, they have a vital effect on its properties. Nor is the starch granule homogeneous. The phosphoric acid, for example, exists very largely in the amylopectin rather than the amylose. Sherman and Baker (*15*) found the amylopectin of potato to contain 0.156 percent P_2O_5 and amylose only 0.023 percent.

Numerous investigators have studied the effect of these different noncarbohydrate constituents on the physical properties of the starch. Samec (*12*) finds that the viscosity of his amylopectin is determined by its phosphoric acid content. Different cations produce very marked differences in the appearance and viscosity of the ester. Hydrogen produces a nearly transparent solution. The alkalies produce translucent, highly viscous solutions, while the alkaline earths give rise to opaque and much less viscous solutions.

The ease with which one cation will replace another in the raw starch granule has suggested this as a possible cause of the differences in the viscosity of different samples of starch of the same origin. Tryller (*17*) found that the salts contained in the tap water used in the manufacture of the starch caused nearly all of the potassium to be replaced by calcium. One of the authors (*9*) was able greatly to change the viscosity of edible-canna and potato starches by shaking with hundredth equivalent salt solutions. The valence of the cation was the determining factor, and a remarkable similarity of properties occurred within any one valence. That replacement was progressive, and in no instance complete, was shown by the continuous change in properties with the number of treatments and variations in concentration of the salt solutions.

The degree of base saturation of the amylophosphoric acid has been studied as a possible criterion of the differences in viscosity. Tryller (*17*) expressed the ratio of cations to phosphorus in terms of equivalent bases per gram atom of phosphorus and found the ratio to vary from 1.19 to 1.85. A ratio of 1.67, he found, produced a neutral starch. Samec (*11*) stated that the titrable acidity allowable for potato starch is 6 cc of N/10 solution of sodium hydroxide per 100 g of starch. One of the authors (*9*) found that in a limited series of starches the least saturated of the potato and canna starches were the strongest, but the valence of the cation was taken to be the more dominant factor, since a strong starch can be produced if it is saturated with monovalent cations.

The entire problem of the relationship between the electrolytes and viscosity of the starch granule is thus in a very unsettled state.

There is a paucity of analytical data from which to draw conclusions. Most of the work thus far conducted has been of a fundamental nature, in which the properties of the specially purified fractions have been studied. The importance of starch in the industrial world warrants more intensive investigation of the relationship of the electrolytes of the starch granule to viscosity, this property being selected because of its very practical importance in most of its industrial uses.

In previous work at this station, the line of attack has been somewhat different from that of other investigations in that the properties of the whole or unbroken granule have been studied, whereas

in most work the starch granule has been broken down and studied as a heterogeneous mixture or fractionated into its components.

The investigation reported in this paper consisted primarily of the determination of the variation of the phosphoric acid and cations in starches of various origins, the nature of their bonding, by means of electrodialysis and salt replacement, and the possible significance of these results in practical industrial uses of starch, as well as in plant chemistry. Circumstances have forced the abandonment of this line of investigation, but the results are presented with the hope that some of the promising lines of investigation may be continued by other workers in this field.

METHODS

ASHING OF STARCH

It was found impossible to ash starch by direct ignition, the difficulty encountered being that the sample tended to fuse into a hard black mass upon loss of its constituent water. Subsequent and long-continued heating failed to produce a light-colored ash. The alcohol-and-glycerin ignition method employed in the ashing of wheat flour proved to be too difficult to control because of frothing. The wet digestion method, using concentrated sulphuric and nitric acids in a tall beaker, was found to be too tedious and the large amount of acids required gave rise to "blanks" which were excessively high.

The following method was finally adopted as being the most practicable:

Twenty grams of starch was placed in a 100-ml platinum dish, and 10 ml of sulphuric acid (specific gravity 1.84) was added. If charring failed to start after 2 or 3 minutes of stirring, the dish was heated slightly near one side, after which the reaction became exothermic and proceeded with a vigorous liberation of steam and sulphur dioxide. The stirring was continued during the reaction to prevent the formation of large gas pockets which might have caused the sample to overflow. After the reaction had ceased, the charred mass remained porous and ashed well at a dull red heat. The ash was taken up with hydrochloric acid and the silica dehydrated by the official method.

PREPARATION OF STARCH

The method used for locally prepared starches was the usual one of grinding or shredding the rootstock or tuber, followed by extraction of the starch from the pulp held on a fine screen or cheesecloth. The starch was purified by repeated stirring, settling, and decanting, ordinary tap water being used unless otherwise stated.[3] The starches were air-dried over a hot plate at a temperature not exceeding 40° C.

Relative viscosity was determined by the method previously outlined (*9*), which consisted in bringing the starch into solution by means of hot water delivered from a pipette. The viscosity was measured in a Stormer viscosimeter and calculated as relative viscosity by reference to a standard curve.

Electrodialysis was carried out in a 3-compartment cell of the type described by Humfeld and Alben (*5*).

[3] The tap water of the city of Honolulu is of excellent quality and seldom exceeds 5 grains per gallon of salts, which are chiefly the chlorides of sodium and calcium.

ANALYTICAL METHODS

Determination of phosphate was made by the Bell-Doisy colorimetric method (*2*).

Several methods for determining calcium were tried but were found unsuitable. Among these were Lyman's nephelometric (stearate) method (*6*), a micro method by Mazza and Rossi (*7*), and a tungstate colorimetric method by Astrug, Mousseron, and Bouissou (*1*). The method finally chosen was gravimetric, the calcium being precipitated in oxalic acid solution (*4*), filtered, dried at 100° C., and weighed as the oxalate (*14, p. 91*). This procedure offers two advantages over that of igniting the residue to calcium oxide: (1) It results in a much greater weight of material, which is of especial importance for the small amount of calcium present; and (2) it permits the whole procedure to be handled in sintered-glass crucibles. The method was tried out on a "known" solution which contained other electrolytes in amounts calculated to give it about the same composition as the ash of starch. Table 1 shows the results obtained.

TABLE 1.—*Estimation of small known quantities of calcium by weighing as the oxalate ($CaC_2O_4.H_2O$) dried at 100° C.*

Ca present	Ca found	Error	Ca present	Ca found	Error
Milligrams	*Milligrams*	*Percent*	*Milligrams*	*Milligrams*	*Percent*
1.795	1.700	−5	1.339	1.289	−4
1.786	1.728	−3	.893	.932	+4
1.346	1.262	−6			

Magnesium was determined by the Bell-Doisy (*18*) method. Potassium was determined gravimetrically by the chloroplatinate method. Sodium was determined gravimetrically as sodium-zinc-uranyl acetate.

ASH CONSTITUENTS OF PRINCIPAL COMMERCIAL STARCHES

A series of commercial samples of starch were secured from a starch broker. These samples were selected to represent the normal run of starches as they are received by the starch broker from different sources. In addition, a number of laboratory samples of edible-canna and other starches were prepared.

A determination of the principal electrolyte constituents was made in the above series of starches. Table 2 gives the results of the analyses.

If we may consider as significant a single analysis of a given kind of starch, certain similarities and dissimilarities between the different starches are apparent. Arrowroot is notable for its extreme paucity of phosphate, which, together with a high calcium content, gives this starch such an abnormal excess of bases present as to indicate the presence of other anions probably existing as occluded salts. Its extremely low magnesium content again brings us to the conclusion that it is the most unusual of this group. Cassava, corn, sago, and sweetpotato starches are very low in phosphate as compared with either potato or canna starches. Rice starch is worthy of notice for its high content of magnesium, and a sodium content which is five times that of the next highest. Wheat starch is the only one of the first group in which the electrolyte content is comparable with that of potato starch.

TABLE 2.—*Ash constituents (percentage) of principal commercial starches*

Sample no.	Kind of starch	Source [a]	PO_4	Ca	Mg	K	Na	Relative viscosity	Ratio total bases [b] PO_4
13	Arrowroot	Commercial	0.015	0.080	0.002	0.012	0.009		30.90
16	Cassava	do	.041	.011	.003	.010	.012		3.66
15	Corn	do	.046	.017	.008	.012	.018		5.34
17	Rice	do	.161	.010	.024	.026	.125		5.07
19	Sago	do	.036	.018	.003	.034	.014		6.90
43	Sweetpotato	Laboratory	.048	.006	.005	.017	.006		2.76
12	Wheat	Commercial	.176	.027	.008	.036	.025		2.19
9	Potato	Commercial, domestic.	.185	.032	.008	.067	.008	0.72	2.22
57	do	do	.154	.015	.006	.023	.009	.72	1.38
11	do	Commercial, Dutch	.255	.026	.010	.019	.015	.85	1.23
86	do	do	.247	.010	.009	.061	.014	1.20	1.32
10	do	Commercial, German.	.171	.047	.009	.023	.011	.90	2.31
14	do	do	.156	.049	.010	.019	.009	1.01	2.52
53	do	Commercial, unknown.	.189	.043	.010	.025	.008	1.00	2.01
54	do	do	.221	.028	.013	.025	.025	1.16	1.83
55	do	do	.152	.020	.021	.013	.013	.81	2.28
66	do	Laboratory, upland—wet.	.173	.017	.021	.025	.016	1.13	2.16
Average for potato.			.190	.029	.012	.030	.013	.95	1.93
Maximum			.255	.049	.021	.067	.025	1.20	
Minimum			.152	.010	.006	.013	.008	.72	
4	Edible canna	Laboratory, lowland, irrigated.	.128	.002	.019	.018	.019	.89	2.19
5	do	Laboratory, lowland, dry.	.140	.014	.016	.010	.020	.88	2.10
20	do	Laboratory, upland.	.147	.011	.022	.018	.024	1.08	2.49
52	do	Commercial [c]	.137	.005	.014	.021	.010	1.00	1.65
63	do	Laboratory, upland, dry.	.136	.010	.016	.019	.016	.92	2.10
64	do	Laboratory, lowland, dry.	.112	.006	.014	.021	.010	.85	2.07
89	do	Laboratory, upland, wet.	.137	.008	.015	.025	.022	1.23	2.31
Average for edible canna.			.134	.008	.017	.019	.017	.98	2.13
Maximum			.147	.014	.022	.025	.024	.123	
Minimum			.112	.002	.014	.010	.010	.85	

[a] All "commercial" samples were secured through the courtesy of Morningstar, Nicol, Inc., New York, who submitted them as typical of commercial starches; all laboratory samples were prepared at the Hawaii Agricultural Experiment Station, Honolulu, Hawaii.
[b] Total milliequivalents of bases divided by millimols of PO_4.
[c] This sample from Waimea Starch Co., Waimea, Hawaii.

A comparison of the average electrolyte content of potato and canna starches shows that potato contains more phosphate, considerably more potassium, and a great deal more calcium, but less magnesium and sodium than do the canna starches. The only constituent determined which shows anything like a constant or characteristic value is phosphate, all others vary between much wider limits. In this group of unrelated samples there is no apparent relation between viscosity and the percentage of any constituent determined.

EFFECT OF STORAGE OF EDIBLE-CANNA ROOTSTOCKS AND POTATO TUBERS UPON THE ELECTROLYTES OF THE EXTRACTED STARCHES

Previous work by one of the authors (*9*) has demonstrated that minute amounts of added electrolytes are able to change the physical properties, and also that these same properties are affected by the

interval that elapses between the digging of the edible-canna rootstocks and the extraction of the starch. It was therefore considered possible that the change occurring during storage of the rootstock is primarily an exchange of cations between sap and starch, which is responsible for the concurrent change in viscosity.

Table 3 gives the effect of storage of edible-canna rootstocks and potato tubers upon the electrolytes of the extracted starches.

TABLE 3.—*Effect of storage of edible-canna rootstocks and of potato tubers upon the electrolytes of the extracted starches, expressed in milliequivalents per 100 g of starch*

EDIBLE-CANNA STARCHES

Sample no.	Time of storage	Phosphate (PO_4)	Calcium (Ca)			Magnesium (Mg)		
			Total	Dialyzable	Proportion dialyzed	Total	Dialyzable	Proportion dialyzed
	Days				*Percent*			*Percent*
89	1	4.27	0.41	0.01	2.4	1.23	0.10	8.1
90	6	4.37	.85	.29	34.1	1.64	.36	22.0
91	15	4.93	.99	.28	28.3	1.80	.40	22.2
92	30	5.00	1.27	.57	44.9	2.05	.45	22.0
POTATO STARCHES								
66	1	5.40	0.85	0.59	69.4	1.72	0.55	32.0
67	5	4.77	1.30	.34	26.2	1.89	.39	20.6
68	17	5.36	1.06	.50	47.2	1.89	.30	15.9
70	80	4.96	1.10	1.03	93.6	1.97	.18	9.1

EDIBLE-CANNA STARCHES

Sample no.	Time of storage	Potassium (K)			Sodium			Ratio of dialyzed monovalent to total dialyzed cations	pH of starch	Relative viscosity
		Total	Dialyzable	Proportion dialyzed	Total	Dialyzable	Proportion dialyzed			
	Days			*Percent*			*Percent*			
89	1	0.64	0.64	100	0.99	0.46	46.4	0.91	7.09	1.23
90	6	.15	.13	86.6	.74	.42	56.7	.46	6.95	1.09
91	15	.15	.12	80.0	.65	.44	67.7	.45	7.08	1.05
92	30	.23	.11	47.8	.65	.44	67.7	.35	7.30	.95
POTATO STARCHES										
66	1	0.64	0.08	12.5	0.70	0.39	55.7	0.29	6.98	1.13
67	5	.49	.09	18.4	.52	.39	75.0	.40	6.98	1.35
68	17	.56	.09	16.1	.48	.35	72.9	.36	7.07	1.27
70	80	.67	.08	11.9	.61	.30	49.2	.24	7.25	1.29

In the canna series several changes occurred that were due to storage. The increase in phosphate was sufficiently large and regular to appear significant. The loss of monovalent cations was nearly equal to the increase in calcium and magnesium. The rate of change was greatest between the 1-day and 6-day storage period, the relative viscosity being reduced from 1.23 to 1.09. The abruptness of the initial drop might be taken as a strong indication that it was caused by the shifting of electrolytes toward a new equilibrium.

The potato series showed significant changes in total electrolytes and a remarkable increase in viscosity between the 1-day and the 5-day storage period. Large as the increase was, there is no reason why it should be assumed to be at its maximum after 5 days, since only four storage periods were investigated. Although the change in electrolytes indicates that the same forces were present as in the canna series, the magnitude of the change produced was less.

Inasmuch as the viscosity increased in spite of the increase in divalent and decrease in monovalent cations, it may be safely assumed that some other very potent force was present. If the amounts of cations dialyzed are considered as representing the active portion of the bases present, the members of the canna series exhibited toward viscosity approximately the same relationship as did total cations. By this method, however, the members of the potato series are brought into much better agreement with their respective viscosities. Sample 70 may safely be omitted in view of the fact that many of the tubers were rotting at the time the starch was extracted.

The considerable differences between the two series in the extent of replaceability of the various cations are shown in the column "proportion dialyzed." In view of the dissimilar natures of the canna- and potato-storage organs, it is remarkable that in the case of both magnesium and sodium, the proportions dialyzed have so nearly the same limits of variation in the two series.

The ratio of monovalent to total dialyzable cations exhibits toward viscosity an acceptable correlation within an individual series.

In these starches viscosity does not appear to be a function of hydrogen-ion concentration. In this study of active bases, uncooked whole granules were used, and every effort was made to preserve their original structure. The discontinuance of this project prevented a similar investigation of the active bases in a starch which had been thoroughly disintegrated as in a ball mill.

REPLACEMENT OF BASES BY SALTS OF VARIOUS CONCENTRATIONS

In view of the present importance attached to the relative replaceability of cations in various colloids, it was considered desirable to determine the replaceability of cations in the starch granule. Accordingly, starch samples were shaken with several concentrations of ammonium acetate and sodium chloride.

Table 4 gives the amounts of electrolytes removed from edible-canna starch no. 52 by distilled water, ammonium acetate, and sodium chloride.

For the sake of comparison, the total composition of edible-canna starch no. 52 and the amounts of the cations removed by electrodialysis were included in this table. The treatment consisted in adding 750 ml of the reagent to 100 g of starch and agitating for 4 hours in an end-over-end shaker. After the starch had settled, the liquid was poured off and twice replaced with 150-ml portions of fresh reagent, shaken up, settled, and decanted. The combined liquids so obtained were freed from starch and analyzed. One cannot fail to be impressed by the magnitude of the amounts removed by distilled water alone. This loss of bases to distilled water had previously been observed in the lowering of the hydrogen-ion concentration produced by successive washings (*9, p. 37*). Since all these

starches were washed during the process of their preparation, it is not known whether these percentages represent additional amounts resulting from successive washings or whether more electrolytes are released by the intermediate drying of the starch. The ease with which even very dilute solutions of salts may cause an exchange of cations between the starch and the solution has been recognized in connection with the necessary use of pure water in the commercial preparation of starch (*9, p. 42*).

TABLE 4.—*Electrolytes removed from edible-canna starch no. 52 by distilled water, by solutions of ammonium acetate and sodium chloride of various strengths, and by electrodialysis*

[Results expressed as percentage of starch]

Treatment of starch	Strength of reagent	Phosphate (PO_4)	Calcium (Ca)	Magnesium (Mg)	Potassium (K)	Sodium (Na)
None		0.137	0.0048	0.0140	0.0210	0.0097
Distilled water		(a)	.0005	.0003	.0070	.0020
Ammonium acetate	0.001 E [b]	.014	.0007	.0012	.0145	.0038
	0.01 E	.017	.0014	.0035	.0185	.0053
	0.1 E	.012	.0016	.0110	.0140	.0045
	E	.007	.0022	.0056	.0117	.0064
Sodium chloride	0.001 E	(a)	.0031	.0012	.0166	(a)
	0.01 E	(a)	.0048	.0031	.0210	(a)
	0.1 E	(a)	.0048	.0061	.0202	(a)
	E	(a)	.0033	.0078	.0210	(a)
Electrodialyzed		(a)	.0003	.0018	.0210	.0038

[a] Not determined. [b] "E," equivalent weight.

Ammonium acetate is evidently less effective than sodium chloride in the removal of cations. The most effective concentration is not the same for the two reagents, nor is it the same for all cations.

Salt replacement seems to be more effective than cataphoresis in removing cations from starch, with the one exception of potassium which is completely removed by either method.

RATE OF REMOVAL OF ELECTROLYTES BY CATAPHORESIS

As previously noted, electrodialysis was carried out in a 3-compartment cell, so constructed as to enable the removal of the contents of either chamber. This was done at various intervals and the contents titrated with acid or base to determine the rate and progress of the reaction. Figure 1 shows the rate and extent of removal of electrolyte from edible-canna starch by cataphoresis.

Nearly 80 percent as much base was removed in the first 10 minutes as in a total of 220 minutes. After 1 hour, the rate of removal dropped to a very low and fairly constant rate. The acid ions were removed at a nearly constant rate, decreasing only slightly toward the end. The total amount of anions removed in the 220-minute period was only about 60 percent of the amount of cations removed. Obviously, for any shorter interval, this proportion would be smaller. All the starches exhibited some inclination to migrate toward the anode, and some of them adhered strongly to that membrane, necessitating continuous scraping and stirring.

EFFECT OF VARIATION OF ELECTROLYTES IN PLANT SAP UPON THE STARCH IN VIVO

It is a well-known fact that the composition of the sap of a plant can be greatly changed by variations in the nutrient solution. An excess of one cation, potash, for example, sometimes represses the absorption of the other cations. Since in vitro one cation readily replaces another in the starch granule, the possibility suggests itself of thus changing the cations of the starch grain in vivo.

Edible-canna plants were grown in a series of water cultures in which the calcium and potassium were varied within wide limits while the other ions were kept constant. In one culture, no. 96-6, the concentration of the entire nutrient solution was made one fifth that of no. 96-3. The edible canna is not well adapted to water cultures, and considerable difficulty was experienced in maintaining growth.

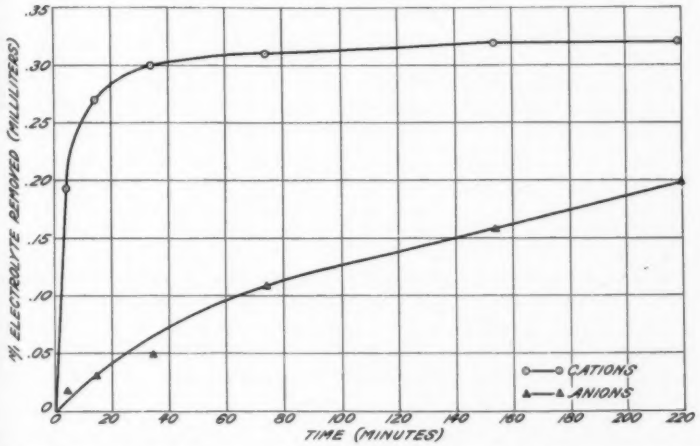

FIGURE 1.—Rate and extent of removal of electrolytes from a 50-g sample of edible-canna starch no. 52 by cataphoresis.

Enough tuber growth was made to secure a small sample of starch from each. Distilled water was used in the washing and purification of the samples. Table 5 gives the composition of the four starches with respect to phosphorus, calcium, and potassium.

TABLE 5.—*Relation of composition of nutrient solution to that of starch from edible canna grown therein*

Sample no.	Concentration of ions in nutrient solution			Concentration of ions in starch		
	PO_4	Ca	K	PO_4	Ca	K
	P.p.m.	*P.p.m.*	*P.p.m.*	*Percent*	*Percent*	*Percent*
96-1	475	391	10	0.057	0.0055	0.005
96-3	475	200	195	.056	.0055	.003
96-5	475	10	381	.056	.0034	.007
96-6	95	40	39	.055	.0041	.006

The results show no tendency on the part of the starch to be affected by the composition of the nutrient solution. The abnormality of the growth is shown in the small percentages of all three ions.

Table 6 gives the difference in electrolytes of starch from edible-canna tubers of a single hill, divided into four groups in different stages of development. A hill of edible canna grows more or less continuously, new rootstocks developing from axillary buds so that at any given time after about 9 months of growth there exist in the same hill rootstocks in all stages of development.

TABLE 6.—*Electrolytes in starch from rootstocks in different stages of development within a single hill of edible canna*

Sample no.	State of development of rootstock	Percentage of indicated electrolyte in the starch				
		PO_4	Ca	Mg	K	Na
71	Group 1, dormant, stalk entirely dead	0.155	0.0212	0.021	0.012	0.014
72	Group 2, mature, stalk full grown	.156	.0206	.022	.009	.014
73	Group 3a, immature, stalk half grown	.199	.0329	.027	.035	.010
74	Group 3b, immature, rapidly developing spikelet	.186	.0240	.028	.032	.010

In general, group 3a gave the highest concentration of electrolytes, potassium showing the greatest relative change. However, considering the fact that the rootstocks in group 1 were about 9 months old, while those in group 3 were just developing, the differences are not great.

Table 7 gives the electrolytes in starch and sap from edible-canna rootstocks stored for varying lengths of time. The sap represents the portion that could be expressed from the pulp by hand, after the tubers had been ground in a meat chopper preparatory to extracting the starch. For the sake of comparison, analyses of the corresponding starches are given.

TABLE 7.—*Electrolytes in starch and sap from edible canna rootstocks stored for varying lengths of time*

Sample no.	Time of storage of rootstocks	Material	Percentage of indicated electrolyte in the starch				
			PO_4	Ca	Mg	K	Na
	Days						
89	1	Starch	.0137	0.0082	0.015	0.025	0.0223
		Sap	.204	.0044	.033	.583	.0030
90	6	Starch	.140	.0171	.020	.006	.0170
		Sap	.239	.0036	.044	.478	.0023
91	15	Starch	.158	.0199	.022	.006	.0150
		Sap	.255	.0048	.050	.671	.0025
92	30	Starch	.160	.0254	.025	.009	.0149
		Sap	.337	.0023	.060	.743	.0026

Table 7 gives strong evidence that there is no direct chemical equilibrium between the plant sap and the ions combined with the starch. Not only is there no correlation between the changes in electrolytes during storage in starch and sap, but the ratio between the percentage of ions in starch and sap for the different ions is a vastly different one. For instance, in sample 89 the starch holds about 8 times as much

sodium as the sap, while the sap holds about 20 times as much potassium as the starch. Treatment of an extracted starch with a salt solution in vitro shows little specificity of cations, an excess of one cation being able to largely replace the other cations (*11*). The very large excess of potassium over the other cations in the sap would be expected to result in far more potassium in the starch than is the case. The greater concentration of calcium and sodium in the starch than in the sap suggests that these ions may be held in a relatively unionized condition. The same may be said of the large increase in calcium with a roughly constant calcium content of the sap. The percentage of the different ions in the starch might thus represent the cumulative effect of various changes in sap concentration during growth or storage, which fact would obscure any relationship between starch and sap at any given time.

While it is evident that the conditions of the foregoing experiments preclude exact and detailed conclusions, they furnish strong evidence that changes in starch electrolytes in vivo are small compared with the effect of similar electrolyte concentrations in vitro. Ionic absorption appears specific and not controlled by the relative concentration of the different ions in the sap. Such selectivity is characteristic of absorption of ions by the living plant cell. The present concept of starch as made up of a carbohydrate, protein, fatty acid, mineral complex adds to the evidence that starch is directly combined with, and affected by, the living protoplasm of the cell.

The extent of the variation in electrolytes caused by differences in climate may be judged by the maximum and minimum values for the different ions given in table 2 for edible canna. Rapid meristematic growth, as compared with dormancy in the same hill (table 6), produces a certain increase in electrolytes. The electrolyte changes taking place during germination (table 3) are of a different order from those of growth, i.e., rapid displacement of potassium, and equally rapid and continuous absorption of calcium to a point where potassium is much lower than the minimum for normal canna starches (table 2), and calcium is far in excess.

Table 8 gives the relative proportions of the ash constituents of the edible-canna tuber present in the starch and nonstarch portion of the tuber.

TABLE 8.—*Proportion of ions held by the starch and nonstarch parts of the edible-canna tuber*

Ion	Edible-canna tuber [a]	Extracted starch	Ions in starch expressed as proportion of tuber [b]	Proportion of ion in starch of ion in whole tuber [c]
	Percent	*Percent*	*Percent*	*Percent*
PO_4	0.646	0.074	0.059	9.1
Ca	.0343	.0027	.0022	6.4
Mg	.205	.012	.010	4.9
K	2.288	.019	.015	.7
Na	.0820	.0092	.0074	9.0

[a] Air-dry basis.
[b] Column 3 by 80 percent because starch in the oven-dry edible-canna tuber runs about 80 percent.
[c] Data in column 4 divided by data in column 2.

These data show the importance of starch as an inorganic storage material. Particularly is this true of phosphorus which is needed in large amounts during germination and in the very early development of the young plant. Decomposition of the starch granule would bring these mineral constituents into solution, possibly in organic combinations especially needed for early growth. For example, Russell (*10*) states that oxidation of carbohydrates involves the simultaneous formation of a hexose phosphate and probably explains the large amount of phosphorus compounds stored up in the seed.

SUMMARY

The major ash constituents were determined for a series of the principal commercial starches.

Edible-canna rootstocks and potato tubers were stored for various lengths of time, and the starch was extracted. The viscosity and composition of these starches were then determined. With the edible-canna series it was possible to show that the drop in viscosity was correlated with a drop in potassium and an increase in calcium. The same changes in electrolytes could not be established in the potato series. In view of the contradictory nature of these results, it was concluded that some method of evaluating the ions according to their activity might give more concordant results. For this purpose electrodialysis and salt-replacement methods were used. Electrodialysis did not change the relationship for the canna series but did assist in bringing the potato series into better agreement.

Salt replacement appears to be more effective than cataphoresis in the removal of bases, but less reliable in the matter of obtaining duplicates.

Significant amounts of cations are removable by shaking with distilled water.

Study was made of the effect of the changes in the electrolytes in plant sap on the starch in vivo. The results showed that while the electrolytes held by the starch are affected to some extent by differences in plant sap and growth conditions, the effect is small compared with the corresponding effect on the starch in vitro. Electrolyte changes in starch due to storage of the rootstocks are larger than those produced during normal growth.

It was concluded the electrolytes occurring in starch in vivo are largely determined by the protoplasm of the plant cell rather than by direct chemical equilibrium with the electrolytes of the plant sap.

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